Ongoing domestication: *in situ* conservation and microevolution of a traditional barley variety (*Hordeum vulgare* ssp.*vulgare*) in northern Morocco.

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DEDICATION

I dedicate this thesis to my son, Arthur Brunelle,
who has transformed and enriched my life.

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PREFACE

Thesis format

This thesis has been writen using a manuscript-based format, in accordance with McGill regulations. Chapters 1, 2, and 5 have already been published in scholarly journals. Chapter 4 is accepted for publication and Chapter 3 is in preparation for submission. References have been formatted using the style of *The New Phytologist*. Manuscript titles and citations are as follow:

Chapter 1: Meyer RS, DuVal AE, Jensen HR* 2012. Patterns and processes in crop domestication: an historical review and quantitative analysis of 203 global food crops. *The New Phytologist* **196**: 29–48. *All authors contributed equally to this manuscript.

Chapter 2: Jensen HR, Belqadi L, De S, Paola, Sadiki M, Jarvis DI, Schoen DJ 2012. A case study of seed exchange networks and gene flow for barley (*Hordeum vulgare* ssp. v*ulgare*) in Morocco. *Genetic Resources and Crop Evolution,* doi.org/10.1007/s10722-012-9909-4

Chapter 3: Jensen HR, Sadiki M, Schoen DJ. Loss of genetic structure in a traditional barley variety in Morocco: the effect of market seed? (For submission to: *Conservation Biology*).

Chapter 4: Jensen HR, Dreiseitl A, Sadiki M, Schoen DJ. High diversity, low spatial structure and rapid pathotype evolution in Moroccan populations of *Blumeria graminis* f.sp. *hordei. European Journal of Plant Pathology*, doi:10.1007/s10658-013-0166-y

Chapter 5: Jensen HR, Dreiseitl A, Sadiki M, Schoen DJ 2012. The Red Queen and the seed bank: pathogen resistance of *ex situ* and *in situ* conserved barley. *Evolutionary Applications* **5**: 353–367.

Contributions of authors

This thesis represents the results of my own research and intellectual work. My supervisor, Dr. Daniel Schoen, is a co-author on Chapters 2,3,4 and 5. He contributed to experimental designs, analysis and presentation of results, and edited those chapters.

Chapter 1 is co-authored by Dr. Rachel S. Meyer and Ms. Ashley DuVal. This chapter presents a review and quantitative analysis of domesticated crops. All three authors contributed equally to the design, data collection, data analysis and writing of the manuscript. This was a highly collaborative effort, and all authors had input on all sections of the paper. However, I was responsible for the sections regarding Life cycle, Ploidy level and Reproductive strategies. Rachel Meyer was responsible for the sections regarding the domestication syndrome and utilization of plant parts and she produced figure 4, Ashley DuVal was responsible for the section regarding spatial and temporal trends, produced Figure 3 and designed the website for the data: www.cropdomestication.com.

Chapters 2, 3, 4 and 5 are co-authored by Dr. Mohammed Sadiki, who was my supervisor while I was doing field and lab work in Morocco, at Institut Agronomique et Vétérinaire Hassan II. He assisted in the design of experiments for all of these chapters. In particular, he contributed to the design of the household survey questionnaire used in Chapter 2 and to the training of the interviewers who administered the survey.

Chapters 4 and 5 are co-authored by Dr. Antonín Dreiseitl. For chapter 4, he contributed the data for isolates of *Blumeria graminis* f.sp. *hordei* from Moravia in 2009. These data were used for the purpose of comparing the isolates I collected in Morocco in 2009 to a contemporary collection from Europe. He also assisted in experimental design and data analysis for this chapter and edited the manuscript. For chapter 5, he characterized the resistance (R)–genes present in the samples of barley varieties collected in 1985 and 2008 using a collection of reference isolates of *Blumeria graminis* f.sp. *hordei* that he maintains in his laboratory in the Czech Republic. Prohibitions on transporting pathogen isolates between countries precluded me from doing this work in Morocco. He also contributed to data analysis and edited this chapter.

Chapter 2 is also co-authored by Dr. Loubna Belqadi L, Ms. Paola De Santis, and Dr. Devra Jarvis. Loubna Belqadi contributed to the design of the household survey questionnaire and to the training of the interviewers who administered the survey. She also edited the manuscript. Paola De Santis contributed to the design of the household survey questionnaire and to the training of the interviewers who administered the survey. Devra Jarvis contributed to the design of the household survey questionnaire and edited the manuscript.

Statement of originality

The use and conservation of agricultural biodiversity is a major preoccupation, particularly in the face of environmental degradation and climate change. In this thesis I first study the domestication history of crops globally, by compiling what is, to my knowledge, the most comprehensive set of crop domestication histories published to date. Although many authors have proposed theories and generalisations describing domestication, these have never been tested against a large and global data set. Using the compiled data, I test established theories and also detect new patterns. In particular, I demonstrate that many domestication syndrome traits considered as common are, in fact, exceptions, including loss of shattering, transitions from outcrossing to self-fertilizing breeding systems and ploidy changes in sexually reproducing crops. I also demonstrate that the time required to domesticate a crop has decreased since the first domestication events and that domestication rates remain high even in the present day, due largely to efforts of breeding institutions. Most importantly, I demonstrate that the domestication syndromes of most crops have evolved over several millennia and continue to be modified to meet changing needs and conditions. This provides a conclusive demonstration of the ongoing and dynamic nature of plant domestication and illustrates that interactions between farmers and the crop varieties they cultivate will continue to shape the evolution of crop traits.

In chapters 2-3, I study spatial and temporal dimensions of diversity in one specific crop, barley in Northern Morocco. Although many authors have described genetic diversity and structure of traditional crops and others have described farmer perceptions of genetic diversity and seed movement through

seed exchange networks (e.g., Soleri and Cleveland, 2001; Pautasso et al., 2012), it is preferable to assess both genetic structure and seed exchange patterns concurrently. Here I provide one of the few studies to take this approach (e.g., Delêtre et al., 2011) and I provide the first comprehensive description of seed exchange networks for barley in this region, coupled with measurements of genetic diversity, structure and gene flow among populations of a complex of local barley varieties. I demonstrate that farmers in this region value traditional barley varieties because of a suite of traits including productivity, local adaptation, drought resistance, and quality of animal fodder that are consistent with their priorities for this crop. I also determine that the majority of seed used for planting the crop is conserved on-farm, with some gene flow from local markets. I also provide what is, to my knowledge, the first example of an assessment of temporal changes in genetic structure of a traditional crop variety over an extended number of years.

In chapters 4-5, I study the evolution of disease resistance in barley to the co-occurring fungal pathogen: *Blumeria graminis* f.sp. *hordei.* Several publications, including important policy documents (e.g., United Nations 1992; Secretariat of the Convention on Biological Diversity, 2010) state that crops conserved *in situ* will have improved local adaptation to co-occurring pathogens compared to crops conserved *ex situ*. However, data from studies of plant-pathogen interactions from wild species suggest that, in some cases, this may not occur, due to fitness costs of resistance alleles and genetic limits to the accumulation of resistance genes (Wei et al., 1999; Cruz et al., 2000; Wei et al., 2002; Burdon and Thrall, 2003; Tian et al., 2003). However, I am the first author,

to my knowledge, to have conducted an empirical test of this assumption using *in situ* and *ex situ* samples of a crop.

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I would like to thank my supervisor, Daniel Schoen, who has helped me to develop as a scientist through his unwavering support, advice, and encouragement during these past years. Thank you for giving me the opportunity to pursue my passion for research in the field of crop genetic resources. I am also very thankful to have had a wonderful supervisor for my field work in Morocco, Mohammed Sadiki at Institut Agronomique et Vétérinaire Hassan II, who instantly made a welcome member of his research team and taught me many valuable lessons, not only about research, but also about project management and administration. I would also like to thank Devra Jarvis at Bioversity International for including me in this stimulating project and for helping to sow the seeds of what has become a very enriching research collaboration with Institut Agronomique et Vétérinaire Hassan II. Thank you as well to my committee members: Jacquie Bede, Ajjamada Kushalappa and Timothy Johns. Jacquie, in particular, thank you for being so supportive, helpful and encouraging in all aspects of this work. For everything from advice on my lab work, inviting me as a guest lecturer in your plant science class, and adopting me as an honorary member of your lab.

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For providing the barley seeds and near-isogenic lines that were essential to this study I thank Kenneth Street at the ICARDA seed bank, Antonín Dreiseitl (Agricultural Research Institute Kroměříž Ltd), Fredrik Ottosson (Nordic Genetic Resource Centre), Harold Bockelman (National Small Grains Collection, USDA) and M. Hilali (INRA, Morocco). Thank you also to Antonín Dreiseitl for so generously training and advising me on all topics relating to phytopathology.

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Thank you to my mother, Kathryn Billane, and my father, Lin Jensen, for being such wonderful role models for independent thinking, and always encouraging and supporting me in all my endeavours. Thank you also to my mother for all the help with childcare that allowed me to finish writing my thesis. And finally, thank you to my wonderful husband, Jérôme-Antoine Brunelle, who has supported me and believed in me through all the ups and downs of my Ph.D. program.

ABSTRACT

Recent evidence indicates that the domestication of crop plants is a protracted and ongoing process, with changes in selection pressure (e.g., selection for a particular trait, cultivation in a new environment, disease pressure) resulting in microevolutionary changes. The interaction between humans and crops is a major driver of crop plant evolution, with a strong influence on gene flow and selection. For this reason, conservation of crop varieties *in situ* (on-farm) is considered important to maintain the adaptation of crop varieties to a diversity of different environmental conditions, cultural requirements, and emerging disease strains. In this dissertation, I study populations of an inbreeding cereal crop, barley (*Hordeum vulgare* ssp. *vulgare*), under farmer management in northern Morocco. I used a combination of household surveys and genetic data to determine the use and valuation of a traditional variety, *Beldi*, and the amount of gene flow among villages and rural communes. *Beldi* is a variety that is valued because of traits that are appropriate to the region (e.g., high productivity in marginal conditions, high quality vegetative biomass) and that has been cultivated for several decades. Levels of gene flow are high both within and among villages and communes, likely due to the influence of local markets. I then studied the genetic diversity and structure of *Beldi* in the region on a temporal scale, by comparing samples of seed collected on-farm in 1985 and in 2008, and also by assessing the seed available in local markets. Genetic diversity has remained high between 1985 and 2008, but genetic structure was not detectable in 2008, likely due to gene flow

from markets to farms. However, seed maintained on-farm is not identical to seed purchased in markets, indicating that on-farm conservation is maintaining a separate pool of local biodiversity. These local populations therefore have the potential to evolve and maintain adaptation to changing local conditions. I then studied the adaptation of *Beldi* to a fungal pathogen endemic to the region: *Blumeria graminis* f.sp. *hordei*, the causative agent of powdery mildew. *B. graminis* is a rapidly evolving biotrophic pathogen, with new virulence genes and virulence gene combinations arising frequently and spreading rapidly across broad geographic regions. Therefore, populations of *Beldi* maintained on-farm could be expected to have higher resistance to the current population of *B. graminis* than sampled collected and maintained *ex situ,* in genebanks. I first characterized the virulence genes present in *B. graminis* populations in the study region and determined that there have been significant changes in the pathogen population during the past two decades. I then assessed qualitative and quantitative resistance of populations of *Beldi* maintained *in situ* and populations maintained *ex situ* to isolates of *B. graminis* from the study region. Although some populations maintained *in situ* had higher resistance, there were also some cases where *ex situ* populations were more resistant. This is likely because, in some cases, *ex situ* populations retain rare resistance genes no longer present *in situ* and to which the pathogen may have lost virulence. This indicates that both methods of conservation are complementary. Overall, this dissertation provides an in-depth study of the interaction between a farming community and a crop, with implications for long-term conservation of crop genetic diversity.

RÉSUMÉ

Des données récentes indiquent que la domestication des plantes de culture est un processus graduel et continu. Des changements dans la sélection naturelle (ex. la sélection pour un trait particulier, un changement dans le lieu de culture, des maladies fongiques) peuvent avoir comme conséquences des changements à l'échelle de la microévolution. L'interaction entre les humains et les espèces cultivées est un des engins majeurs de l'évolution de ces espèces, avec une grande influence sur le flux des gènes et la sélection. Pour cette raison, la conservation des variétés de culture *in situ* (à la ferme) est considérée comme une stratégie qui peut maintenir l'adaptation des variétés à une diversité de conditions environnementales, de milieux culturels, et de maladies émergentes. Dans cette dissertation, j'ai étudié des populations d'une plante céréalière autoféconde, l'orge (*Hordeum vulgare* ssp. *vulgare*), qui sont gérées par des agriculteurs dans le nord du Maroc. J'ai fait usage de questionnaires auprès des agriculteurs ainsi que de marqueurs génétiques pour déterminer l'usage fait et la valeur attribuée à une variété traditionnelle, appelée *Beldi,* ainsi que pour quantifier le flux de gènes entre les populations au niveau des villages et des communes rurales. La variété *Beldi* est valorisée par les agriculteurs parce qu'elle possède des caractères qui la rendent appropriée pour la région (ex. haute productivité dans des conditions marginales et haute qualité de la biomasse végétative) et, en conséquence cette variété est cultivée depuis plusieurs décennies dans la région. Il y a un haut taux de flux de gènes entre les villages et entre les communes, probablement facilité

par les marchés locaux. Par la suite, j'ai étudié la diversité et la structure génétique de *Beldi* dans la région sur une échelle temporelle, en comparant des échantillons de semences collectés chez les agriculteurs en 1985 et en 2008, ainsi que des échantillons des semences disponibles dans les marchés locaux. La diversité génétique est demeurée élevée entre 1985 et 2008, mais nous n'avons pas détecté de structure génétique en 2008. Ceci est probablement attribuable à un haut niveau de flux de gènes, possiblement incluant le mouvement de gènes des marchés locaux vers les champs agricoles. Par contre, les semences maintenues chez les agriculteurs ne sont pas identiques aux semences disponibles dans les marchés, ce qui indique que la conservation des semences à la ferme a comme résultat de maintenir une réserve de biodiversité locale. Ces populations locales ont donc le potentiel d'évoluer et de maintenir une adaptation aux conditions locales changeantes. Par la suite, j'ai étudié l'adaptation de *Beldi* à un agent pathogène fongique qui cause la maladie de l'oïdium et qui est problématique dans la région: *Blumeria graminis* f.sp. *hordei*. *B. graminis* est un champignon biotrophe qui évolue rapidement en acquérant des nouveaux gènes de virulence ainsi que des nouvelles combinaisons de gènes de virulence. Ces nouvelles virulences peuvent être disséminés rapidement dans la population de *B. graminis* sur des grandes régions géographiques. En conséquence, les populations de *Beldi* maintenues chez les agriculteurs et qui sont continuellement exposées à la population de *B. graminis* pourrait avoir une meilleure résistance que des échantillons maintenus *ex situ*, dans des banques de semences. J'ai caractérisé les gènes de virulence présents dans les populations de *B. graminis* dans le nord du Maroc et j'ai déterminé qu'il y a eu des changement significatifs de virulence

dans les deux dernières décennies. Par la suite, j'ai quantifié la résistance qualitative et quantitative de populations de *Beldi* issues de conservation *in situ* et *ex situ* dans la région à des populations de *B. graminis*. Certaines populations maintenues *in situ* étaient plus résistantes que des populations *ex situ.* Par contre, il y avait aussi certains cas ou la population *ex situ* avait une plus haute résistance. Une explication possible pour ce phénomène est que, dans certains cas, la population *ex situ* a retenu des gènes de résistance rares qui ne sont plus présents *in situ* et auprès desquels le pathogène n'a plus de virulence. Ceci indique que les deux méthodes de conservation sont complémentaires. En conclusion, cette dissertation fourni une étude de l'interaction entre des agriculteurs et une espèce agricole, avec des implications pour la conservation à long terme de la biodiversité agricole.

GENERAL INTRODUCTION

The food crops that feed the growing global population are all the result of the domestication of a phylogenetically diverse group of wild plant species via a series of domestication syndromes that have enhanced their palatability, reduced toxicity and increased ease of cultivation and harvesting, among others (Harlan, 1971; Harlan, 1992; Purugganan and Fuller, 2009). In recent years, there has been debate regarding the time scale of domestication, with some researchers arguing for a rapid transition to domestication over decades or centuries (Gepts, 2004), based on molecular data and simulated selection experiments. Currently, most data support a protracted transition to domestication over hundreds and thousands of years (Fuller, 2007; Allaby et al., 2008; Fuller et al., 2012). However, most studies have only studied a narrow subset of crops, either from a specific geographic region (e.g., Harlan, 1992) a specific phylogenetic grouping (e.g., Glemin and Bataillon, 2009) or a specific category of crops (e.g., Miller and Gross, 2011). In the introductory chapter of this thesis, I compile a large dataset representative of global food crops to test current theories of crop domestication.

In situ conservation of crop genetic resources refers to the maintenance of crop biodiversity in agricultural fields by the communities who have used and developed traditional crop varieties for many centuries (Brush, 1995). This is in contrast to *ex situ* conservation of crop varieties where representative samples of plant populations are stored for long periods in seedbanks and thus unexposed to agricultural conditions and their attendant evolutionary effects (Frankel, 1974). *In*

situ conservation of crop germplasm mostly occurs *de facto*, independently of conservation programs, through the ongoing use and selection of traditional variety populations by farmers (Brush, 2004; Bisht et al., 2006; Jarvis et al., 2008). A large proportion of agricultural biodiversity is maintained by farmers in the developing world on over 10 million hectares (Altieri, 2004). This crop diversity conserved *in situ* is subject to selection, migration and genetic drift, similar to wild species.

Gene flow between traditional variety populations and regions is a consequence of farmer seed systems (the human networks through which farmers exchange or sell the seeds of traditional varieties) and can affect metapopulation structure by creating sources and sinks of genetic variation (Alvarez et al., 2005). Extinction and recolonization events may also have long-term impacts on crop genetic diversity (McCauley et al., 1995). In addition, natural selection through disease pressure and abiotic stresses can influence the trajectory of traditional variety evolution (Tin et al., 2001). Although *in situ* conservation preserves traditional variety diversity (Teshome et al., 1999) and evolution (Perales et al., 2003), little is known about the impact of *in situ* conservation on the genetic structure and local adaptation of traditional varieties. In my Ph.D. research, Moroccan barley landraces (*Hordeum vulgare* L. ssp. *vulgare*) and the co-evolved fungal pathogen (*Blumeria graminis* (DC.) Golovin ex Speer f.sp. *hordei*) are studied as a representative system to investigate questions pertaining to the impact of *in situ* conservation on traditional variety evolution.

Barley has been a component of traditional agricultural systems in Morocco for eight thousand years (von Bothmer et al., 2003) and the Moroccan

traditional varieties are an important source of genotypic variation in the global gene pool of this crop species (Badr et al., 2000; Yasuda et al., 1993). Currently, 80% of Moroccan barley is cultivated in agriculturally marginal areas (Benhadfa, 1989; Amri et al., 2002; Saidi et al., 2002; Saidi et al., 2006).

Powdery mildew (*Blumeria graminis* f.sp. *hordei*) is a fungal foliar disease that causes average yield losses of 10% to 30% on barley in North Africa (Caddel and Wilcoxson, 1975; Amri et al., 2002). Co-evolution between host resistance and pathogen virulence involves a gene-for-gene system (Flor, 1956) with reciprocal selection on the host (Paillard et al., 2000a; Paillard et al., 2000b) and the pathogen (Wolfe et al., 1992). Selection pressure exerted by the pathogen is higher in North Africa compared with Europe (fungicides are not used to control the disease in North Africa), and the obligate sexual reproductive stage of the disease (imposed by the hot, dry summers) (Wolfe and Schwarzbach, 1978) can result in considerable genotypic variation in the pathogen population (Welz and Kranz, 1987; Bousset and de Vallavieille-Pope, 2003). The research topics in much of my thesis focus on temporal variation in the population genetic structure of barley, and the spatial and temporal dynamics of local adaptation between barley traditional varieties and *Blumeria graminis* f.sp. *hordei.*

This research is an attempt to integrate the study of a human-crop interaction with evolutionary principles and to use the outcomes of the research to propose conservation strategies. This thesis has strong implications for the study and conservation of traditional crop varieites and genes for pathogen resistance both *in situ* and *ex situ*.

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CHAPTER 1: Patterns and processes in crop domestication: an historical review and quantitative analysis of 203 global food crops

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Abstract

Domesticated food crops are derived from a phylogenetically diverse assemblage of wild ancestors through artificial selection for different traits. Our understanding of domestication, however, is based upon a subset of well-studied "model" crops, many of them from the Poaceae family. Here, we investigate domestication traits and theories using a broader range of crops. We reviewed domestication information (e.g., center of domestication, plant traits, wild ancestors, domestication dates, domestication traits, early and current uses) for 203 major and minor food crops. Compiled data were used to test classic and contemporary theories in crop domestication. Many typical features of domestication associated with model crops, including changes in ploidy level, loss of shattering, multiple origins, and domestication outside the native range, are less common within this broader dataset. In addition, there are strong spatial and temporal trends in our dataset. The overall time required to domesticate a species has decreased since the earliest domestication events. The frequencies of some domestication syndrome traits (e.g., non-shattering) have decreased over time, while others (e.g., changes to secondary metabolites) have increased. We discuss the influences of the ecological, evolutionary, cultural and technological factors that make domestication a dynamic and ongoing process.

Introduction

It is estimated that 2500 plant species have undergone domestication worldwide, with over 160 families contributing one or more crop species (Zeven and de Wet, 1982; Dirzo and Raven, 2003). Much of our understanding of the processes driving domestication comes from a subset of well-studied crops, particularly crops of major economic importance and model crops (i.e., crops that have had their genomes analyzed and are transformable). These crops have been critical for developing our fundamental understanding of domestication as a continuum of ongoing processes. In particular, they have been critical for revealing the underlying genetic mechanisms responsible for the suite of phenotypic changes associated with domestication that comprise the domestication syndrome. They have also contributed to our knowledge of useful crop breeding traits, such as pathogen resistance, and of fundamental biological processes, such as polyploidization. However, information on such well-studied crops contributes disproportionately to the literature on domestication. In order to explore global trends and historical patterns in domestication, large datasets are required that consider a broad selection of species including understudied crops and crops of minor economic importance in addition to well-studied major global crops. Often, the data relevant to the history of use, selection and domestication of a particular crop are scattered across the literature of diverse disciplines. For many minor food plants, information relevant to domestication history may be difficult to access, if it is available at all. Most reviews have not included all the major agricultural regions but rather have concentrated on regional subsets of crops (Duke and Terrell, 1974; Harlan, 1992), or focused on specific groups including

recent studies of the Asteraceae (Dempewolf et al., 2008) and Poaceae (Glémin and Bataillon, 2009), and on previously neglected groups such as vegetatively propagated crops, perennials, and underutilized crops (McKey et al., 2010; Miller and Gross, 2011; Padulosi et al., 2002; Padulosi et al., 2011).

This review considers information on 203 major and minor crop plants compiled across 36 categories including center of domestication, changes in phenotype and use from the wild to the cultivated forms, uses, exploited organs, and conservation status. The threefold objectives of this paper are (i) to identify and interpret patterns in domestication by identifying trends across numerous categories of data on crop domestication and use; (ii) to test current and classic theories in domestication against this large sample; and (iii) to identify promising areas for further research based on the critical questions and gaps in the literature identified by this study. Data summaries and key analyses are presented and discussed in this review, while more detailed information, further analyses and crop bibliographies are provided as supporting information (Appendix I, Tables S1-S6). Updated versions of crop bibliographies (Appendix I, Tables S2, S4 and S5) are maintained at: www.cropdomestication.com.

1. Historical context

The transition from hunter-gatherer societies to settled agriculture (the "Neolithic Revolution"; Childe, 1949) occurred independently over a dozen times in different regions around the world from ca. 10-12,000 years ago (y.a.) to as recently as 3-4,000 y.a. (Diamond, 2002; Diamond and Bellwood, 2003). Theories to explain the origins and development of agriculture have

considered factors ranging from changes in climate and population expansion to cultural practices and religious beliefs (Harlan, 1992).

Current works continue to explore when, where, why and how wild plants became our modern food crops, while also considering the new technical, ethical and environmental challenges of emerging agricultural technologies (Murphy, 2007; Vaughan et al., 2007; Ellstrand et al., 2010; Thrall et al., 2010; Cuevas-Badallo and Vermaas, 2011; Domingo and Giné Bordonaba, 2011; Ekici and Sancak, 2011). In recent years, scientists have used molecular techniques to test and apply theories of crop origins put forward 150 years ago by Darwin (1868) and De Candolle (1884) (e.g., Doebley et al., 1995; Gepts, 2004; Zohary, 2004; Fuller, 2007; Gregory, 2009; Brown, 2010). New works have increasingly recognized the importance of combining the relevant data from several fields to inform observations on crop domestication (e.g., Kroll, 2000; Nesbitt and Tanksley, 2002; Zeder et al., 2006; Vaughan et al., 2007; Wang et al., 2008; Purugganan and Fuller, 2009; Richards et al., 2009; Meyer et al., 2012).

Although recent innovations are causing drastic modifications to the domestication pathways for many species (Vaughan et al. 2007), domestication has always been a dynamic process. New artificial selection pressures have arisen thoughout the history of crop cultivation driven by many factors including new uses for existing crops (e.g., grain crops adapted for biofuel production) and the movement of crops to new environments. These have continually re-shaped the evolution and geographic distribution of crops over time.

Key concepts and definitions

1. Food crop

To meet our criterion of being a food crop, a plant species must have been used at some time as a food, spice, edible oil, beverage, or fasting aid with nutritional value (e.g., khat). In the cases of some of the crops we selected, food uses are secondary, such as fiber crops with oil seeds (e.g., cotton, flax, hemp).

2. Reproductive strategy

Many wild plant species are characterized by more than one reproductive strategy, including sexual breeding systems (e.g., outcrossing or self-fertilizing), and asexual strategies (e.g., vegetative or clonal propagation). Under cultivation, however, only one of these strategies is usually exploited as a propagation method for a given species. Here, we refer to crop reproductive strategies based on the strategy primarily used under cultivation.

3. Domestication syndrome

The suite of traits that marks a crop's divergence from its wild ancestor(s) is defined as the "domestication syndrome" (Harlan, 1971; Hammer, 1984; Harlan, 1992). A domestication syndrome may include combinations of several different traits, including seed retention (non-shattering), increased fruit or seed size, changes in branching and stature, change in reproductive strategy, and changes in secondary metabolites. The domestication syndrome may evolve over thousands of generations, as desirable traits are selected for in the agricultural environment and become fixed within the crop genome (Fuller, 2007; Fig.1). The domestication syndrome may also evolve within a short time frame, as in the

cases of crops domesticated within the last hundred years or so (e.g., kiwi, cranberry).

Similar domestication traits may arise independently multiple times, often under the control of different genes. For example, the loss of a shattering mechanism of seed dispersal via a brittle rachis has arisen in many crops, particularly grasses (Allaby et al., 2008); in barley, two independent domestication events targeted this trait via two different genes (*Bt1* and *Bt2*) (Takahashi, 1955; Fig. 1).

4. Degree of domestication

Domestication traits arising through artificial selection are desirable to farmers and consumers. For instance, they can ease harvest work and enhance taste and nutritional qualities. Often, domestication selects against traits that increase the plant's defensive or reproductive success in natural environments. Artificial selection can therefore work in opposition to natural selection, and domesticated crops have reduced fitness, or, in some cases, an inability to survive outside of cultivation (Gepts 2004; Pickersgill, 2007; Allaby et al., 2008; Purugganan and Fuller, 2011).

Selection can be unconscious or conscious. In unconscious selection, likely the driver of many early domestications, the act of moving plants from the wild into man-made environments alters selection pressures, leading to increased fitness of phenotypes that have low fitness in the natural environment. Human management, including planting and harvesting techniques, creates further selection pressures (Fuller et al., 2010). In conscious selection, desirable

phenotypes are selected, while less desirable phenotypes are neglected or actively removed until their frequency decreases in the population (Zohary, 2004).

Here, "domesticated" refers more generally to plants that are morphologically and genetically distinct from their wild ancestors as a result of artificial selection, or are no longer known to occur outside of cultivation. We define "semi-domesticated" as a crop that is under cultivation and subjected to conscious artificial selection pressures. Although named cultivars may exist, these are not yet clearly morphologically or genetically distinct from their wild counterparts. Finally, "undomesticated" refers to uncultivated plants that continue to be wild harvested with no conscious artificial selection pressures and no discernible morphological and/or genetic differentiations that could be used to distinguish them as a domesticate (e.g., Brazil nut).

5. Center of domestication

Vavilov defined eight "centers of origin" according to certain criteria: high varietal diversity, co-occurrence of wild ancestors with their domesticates, and a long history of crop use (Vavilov, 1926; 1951; Harlan, 1971; Vavilov, 1992; Appendix I, Table S3). The distribution of crop plants and wild relatives has since proven to be more complex than originally proposed and not all crops exhibit centers of high varietal diversity (Harlan, 1992). Recent archaeological work has further modified the definition of centers of origin, revealing small independent centers of origin within previously established centers. For example, India, originally considered as one center of origin, actually contains five independent centers of origin (Fuller, 2009). Other broad regions are now also recognized as important areas of domestication activity, including Near Oceania (Brandes, 1958;

Allaby, 2007), Amazonia (Clement, 1999a; Clement, 1999b; Clement et al., 2010), Eastern North America (Zeven and Zhukovsky, 1975; Smith, 2006), and the river deltas of Western Africa (Harlan, 1971; Portères, 1976). Therefore, because centers of origin are difficult to define and delimit, we favor the term "center of domestication" to encompass broad areas with domestication activity. These include Vavilov's original eight centers of origin and the four more recently proposed centers.

6. Single versus multiple origins

A crop species has a "single origin" when it was domesticated once from the wild ancestor(s), followed by dispersal. A crop has multiple domestication events, referred to as "multiple origins", when domestication occurred independently, from the same ancestor(s), in different locations or times. A domesticated species with multiple origins can have many forms with different common names (e.g., *Brassica oleracea*; Table 1). In contrast, different species that were independently domesticated from different wild ancestors can share a common name (e.g., Asian and African rice) but are considered many single origin events. Researchers determine the number of origins based on multiple lines of evidence, including archaeological, genetic, and linguistic data. For crops where there is insufficient evidence in the literature to determine the number of origins, the default assumption in this review is of a single origin because this is the most parsimonious explanation. Further research may, however, reveal that some presumed single origin crops did, in fact, have multiple origins.

Methods of review and analysis

1. Selection of species for review

The crop species in this review were selected through a screening of multiple sources including the peer-reviewed literature on domestication and economic botany, literature reviews including reviews of specific categories of crops (e.g., trees), and selected databases (Appendix I, Tables S1- S2). An initial 100 food crop species were identified from these sources to include crops from all continents, to minimize bias towards well-studied and/or familiar areas. There was no consideration for familiarity, importance of the crop, or the amount of data available. We selected an additional 80 species with the added criterion of phylogenetic distribution in plant families to ensure that no single family represented more than 7% of the dataset. If too many crops from a single family were included, we randomly selected species to be removed from the dataset. To avoid bias from disproportionate representation of crops of current major economic importance, we identified an additional 23 crops of minor global economic importance but high local importance in developing regions from the economic botany, domestication, and development literature addressing underutilized crops.

The final dataset is composed of 203 crops spanning 68 families, representing 43% of the estimated number of families in which domestication has occurred (Dirzo and Raven, 2003; Table 1). These species and families were grouped into phylogenetic clades for subsequent analysis based on the most current angiosperm phylogeny (Soltis et al., 2011). This dataset includes a large selection of "minor" crop plants that are locally important. We consider crops as

minor if the area devoted to their cultivation is not included in FAOStat (http://faostat.fao.org). By this criterion, 47% of the dataset crops are minor. Forty-three percent of the crops are considered to be underutilized, based on inclusion in the Global Facilitation Unit for Underutilized Species database (Table 2).

2. Literature review

We collated information on crop domestication history, uses, and specific traits, including ploidy level, reproductive strategy, and lifecycle. Our references are mostly peer-reviewed publications and academic sources but also include some grey literature and well-referenced online databases, particularly for minor and understudied crops (Appendix I, Table S1). In total, we compiled information from over 800 references that have been organized as a table that is searchable by crop (Appendix I, Table S2). We categorized this information using predefined parameters (Appendix I,Table S3). We assigned a confidence score to each crop representing the overall availability and quality of data regarding the place of origin, time of domestication and wild ancestor identity, including how well data were supported with archaeological remains and whether evidence was contested (Appendix I, Tables S4-S5).

Using the FAO World Information and Early Warning System (WIEWS), we compiled data for the conservation status of crops using the number of institutions worldwide housing *ex situ* collections of each crop, and the total number of accessions and subspecies available in these institutions (Table 2, Appendix I, Tables S4-S5). WIEWS records are subject to some imprecision because they are reported on a voluntary basis and are not continually updated. Therefore, we also

tabulated *ex situ* conservation data from the databases of a subset of prominent germplasm centers in the Western hemisphere (NORDGEN, USDA ARS-GRIN and Svalbard) and the network of CGIAR germplasm centers (SINGER) (Table 2, Appendix I, Table S1). Although discussion of trends in *ex situ* conservation are beyond the scope of this review, the results of correlation analyses of *ex situ* conservation status with other domestication traits are presented as Supporting Information (Appendix I, Table S6).

3. Data analysis

The compiled and categorized data for all 203 crops were coded as a binary matrix (Appendix I, Table S4). Our initial analysis of these data used Logic Forest (LF), a package in R statistical software (R Development Core Team, 2011) to identify correlations of interest across the multiple categories of data in the matrix. LF, which performs an ensemble classification of multivariate regressions, was designed to identify predictive variables in large and noisy datasets, and has a superior performance to logic regressions in identifying important predictors (Wolf et al., 2010). We treated data in each category as outcomes with possible predictors (i.e., other variables) and used LF to assign a predictor importance (PI) value representing the strength of either positive or negative correlations among the predictor and outcome variables. Some categories of data were removed from specific analyses if they were obviously redundant or linked (e.g., "diploid" as a negative predictor for "polyploid"; Appendix I, Table S5). Crops with missing data in some categories were excluded from certain analyses. We also excluded undomesticated crops from regressions when testing questions specific to domesticated plants. Results of the LF analysis were compiled in a heat map

showing the strength of positive and negative predictors among the different categories in the dataset (Appendix I, Table S6). These results were used to identify potentially interesting relationships among categories and to guide further exploration of the data using classical statistical tests.

Estimates of the time required for a crop to transition from its wild to domesticated forms were calculated by subtracting the date of the earliest record of its domesticated form from the earliest date of exploitation of the wild ancestor. These data were available for 142 crops, based upon archaeobotanical evidence or written records. This method was used for consistency in determining domestication periods, even though more precise methods have been used, particularly for model crops (e.g., Allaby et al. 2008; Fuller et al. 2011b).

We generated maps of the density of domestication events during different time intervals, overlaid on floristic regions as defined by Takhtajan (1986), using ArcGIS (ESRI, Redlands, CA, USA). Takhtajan's floristic regions were chosen over more fine-grained divisions (e.g., WWF ecoregions) because their scale and boundaries are frequently similar to those of proposed centers of domestication. Plots of the data were made in Microsoft Excel or in R statistical software.

Trends identified from the review of 203 crops

Because domestication is an ongoing process, this analysis included crops in varying stages of domestication. The crops included: 160 domesticated, 37 semi-domesticated and 15 undomesticated crops (some crops fell into two categories, e.g., a crop that is mostly wild-harvested, but also has semi-domesticated populations; Table 2; Appendix I, Tables S3-S5). Crops exhibited a wide distribution within categories including uses, plant organs used, geographic

origins, life history traits, and domestication syndrome traits. Most crops were exploited for several organs and for many different uses. The crop domestication syndrome consisted of 2.8 traits, on average. Eighty-four percent of the crops had between two and five domestication syndrome traits, while some crops were defined by as many as seven (Appendix I, Tables S4-S5). The confidence scores for availability and quality of the data regarding the place of origin, time of domestication and wild ancestor identity reveal major gaps in the literature: just 30% of the crops had high confidence scores (Appendix I, Table S5). Ten percent of the studied crops were under dispute with regard to their origins or wild ancestor.

In the following sections, a series of core topics related to domestication are explored. Both LF regression and an evaluation of the data distribution are applied to re-examine domestication hypotheses and test models from the classic and recent literature, and to explore potential novel patterns and trends. LF output for all categories, including robust trends not discussed in the text, is available as a heat map displaying the PI values for each variable (Appendix I, Table S6). The strongest and most potentially interesting results from our analysis are discussed in sections organized around life cycles, ploidy, reproduction, spatial-temporal trends, and uses.

Life cycle

The cumulative number of annuals domesticated per 1000-year time period reveals that domestication events of annuals increased in number from 9000 to 4000 y.a., with a peak 8000 y.a., followed by a steady increase culminating in a second peak 5000-4000 y.a. (Fig. 2a). The domestication of annuals has since

exhibited a decrease over the past 4000 years (Fig. 2a). This could be because most of the annual plants amenable to domestication in the regions settled by humans had already been domesticated by this time. It could also be explained by the broad dissemination of major annual seed crops (wheat, barley, rice and corn) decreasing reliance on minor grains, leading to the loss of minor domesticates or a reduced need to domesticate more annuals. One example of this is sumpweed, which was domesticated in North America as a seed crop and then abandoned when corn replaced it in local diets 700-1000 y.a (Gepts, 2004).

Biennials appear in the dataset beginning nearly 6000 y.a. and increase in every subsequent 1000-year time period (Fig. 2a). Their later occurrence is consistent with the need for more sophisticated crop management techniques and a sedentary lifestyle, because plants must be reserved for an additional year, rather than harvested, to obtain seeds for propagation (Sauer, 1993). Biennials are associated with the circumboreal floristic region in the LF results. Humans domesticate the plants that are available and amenable to domestication in the region where they live. The circumboreal region is suitable habitat for biennials because many have a life cycle that requires vernalization and are therefore more likely to occur in regions with a distinct winter season (Amasino, 2004). The appearance of domesticated biennials is also positively associated with the Mediterranean floristic region in the LF results, peaking between 3000-1000 y.a., coinciding with the rise of major civilizations in Ancient Egypt, Ancient Greece and the Roman Empire in the Mediterranean basin and in Europe (Fig. 2a, Fig. 3a, Appendix I, Table S5). It is possible that the domestication of many crops with a biennial lifecycle in the Mediterranean region was facilitated by knowledge

dissemination throughout the region via the extensive trade networks that existed among these civilizations.

An increase in domesticated perennials coincides with a decline in the rate of annual domestication (Fig. 2a). These findings are consistent with conclusions by Miller and Gross (2011) that trees and other long-lived perennials (collectively refer to as "trees" throughout this review; long-lived perennials also include banana and palms) were domesticated later than annuals. Few trees were domesticated prior to 4000 y.a., and over 50% of the included tree crops were domesticated in the last 2000 years (Fig. 2a). The number of domesticated tree crops increased in two waves, with the first starting 6000 y.a., with a peak 4000 y.a., and a second wave starting 3000-2000 y.a. and continuing into the present era (Fig. 2a). The two waves of domestication observed in perennials may be linked to the dissemination of propagation techniques. It has been proposed that the first wave in fruit domestication (both non-tree perennials and trees) in the Old World coincides with the domestication of species that can easily be propagated vegetatively using simple techniques such as cuttings or suckers (e.g., olive), while the second coincides with the discovery and dissemination of scion grafting (e.g., carob; Zohary, 2002; Hsina and El-Mtili, 2009). The two waves of domestication for trees coincide with this time frame, particularly the second wave beginning 3000-2000 y.a., which is the time frame for the development of scion grafting techniques in the Mediterranean basin. In fact, of all the trees with a domestication syndrome that featured a change in reproductive strategy to mainly vegetative propagation, 76% were domesticated during one of the two waves (Appendix I, Tables S4-S5).

We tested whether crops with different life cycles exhibited significant differences in domestication rates and in the average number of domestication traits. According to Pickersgill (2007), *"*vegetatively propagated root crops and perennial fruit crops show fewer domestication syndrome traits than annual seed crops, and domestication may occur more slowly because fewer sexual generations occur in a given period of time." We found perennial fruit crops do indeed exhibit significantly fewer domestication syndrome traits than annual seed crops (Table 3). However, vegetatively propagated root crops do not exhibit significantly fewer traits than annual seed crops (Table 3). Regarding the rate of domestication, there was no significant difference in the mean time to domestication between vegetative root crops and perennial fruit crops compared to annual seed crops (Table 3). However, in analyses comparing all trees to annuals, the time to domestication was significantly longer and there were significantly fewer domestication syndrome traits in trees (Table 3), consistent with trends described by Miller and Gross (2011). While this result is reasonable, it is also possible that some traits of the tree domestication syndrome are still uncharacterized, producing biased results.

Ploidy level

Polyploidy has been an important factor in angiosperm evolution, underlying episodes of adaptive radiation in many plant families (Soltis and Soltis, 1999) and in the angiosperms as a whole [see Soltis et al., (2009) and references therein]. It is estimated that 15% of speciation events in the angiosperms involve polyploidization (Wood et al., 2009). A number of traits associated with polyploidy such as larger seed size, increased disease resistance, and decreased

allocation to reproduction are advantageous in domesticates (Lewis, 1980; Levin, 1983). Some crops, like wheat, have been derived from wild ancestors through a process of allopolyploidization, conferring desirable characteristics of both ancestors on the new species. Other crops have been domesticated or improved via autopolyploidization (e.g., potato). Autopolyploids typically have larger cells and organs than their diploid progenitors due to doubling of DNA content, while allopolyploids are variable in this regard (Ozkan et al., 2003). The alteration of plant traits may also confer a fitness advantage in certain habitats, allowing species to adapt to marginal environments beyond the natural range of their diploid ancestor (Ramsey, 2011). This could enable domesticates to adapt to disturbed agricultural environments that are not suitable for the wild ancestor. Furthermore, polyploidization provides a mechanism for sympatric speciation, by providing reproductive isolation of the new species from the co-occurring diploid form (Soltis et al., 2007). This may allow the rapid divergence and fixation of traits that are of interest for cultivation by limiting gene flow between wild and cultivated forms. Despite the importance of increased ploidy in the domestication syndrome of such major crops as wheat and potato, little is known about the frequency and importance of ploidy changes in domestication syndromes across a broader sample of crops.

Of the crops with known ploidy (n = 199), our dataset includes 64% diploid crops and 17% polyploid crops, while 19% of the crops have both diploid and polyploid varieties (Table 2). This last proportion is slightly larger, although comparable, to the number of angiosperm species that include multiple ploidy levels (12-13%; Wood et al., 2009). We examined how many polyploid

angiosperm crops underwent polyploidization during the process of domestication and identified 37 crops (19%). Therefore the frequency of these ploidy changes is similar and only slightly higher than the frequency observed in speciation events among angiosperms, which is 15% (Wood et al., 2009), suggesting that ploidy changes do not distinguish evolution under domestication. Of these, 51% were due to autopolyploidy and 24% were due to allopolyploidy while 10% may have arisen from a combination of both and the remainder were of unknown origin or due to ploidy reduction (Table 2, Appendix I, Tables S4-S5).

Perennial crops were the most common category of domesticates with ploidy changes as a domestication trait (78%). Of these, 90% were mainly propagated vegetatively under cultivation, and therefore would not have suffered decreased reproductive output in the event of genome duplication (Ramsey and Schemske, 2002). This is further supported by the observation that, of the crops with a domestication syndrome involving a ploidy change, nearly half (43%) also exhibited a change in reproductive strategy from outcrossing or self-fertilizing to vegetatively propagated. This is a trend previously noted for fruit trees (Zohary and Hopf, 2000).

The proportion of crops with a domestication syndrome involving both a ploidy change and a reproductive strategy change was significantly higher than the null expectation ($chi^2 = 6.418$, df = 1, $p = 0.011$). A remaining 38% of crops with a ploidy change in their domestication syndrome were already propagated vegetatively in their wild form. Human intervention facilitating the propagation of crops with increased ploidy is also supported by the simultaneous increase of ploidy changes and reproductive strategy changes in crops domesticated between

3000-2000 y.a. This coincides with a wave of domestication of perennials (Fig. 2a,b).

The outcrossing crops in our dataset exhibit a lower frequency of changes in ploidy as a domestication syndrome trait compared to self-fertilizing and vegetatively propagated crops (Appendix I, Tables S4-S5). Furthermore, in the dataset as a whole, only 19% of crops with either a self-fertilizing or an outcrossing reproductive strategy had a change in ploidy occurring under domestication that was not associated with a transition to vegetative propagation (bread wheat, noni, oat, okra, rapeseed, soy, and sumpweed). Model cases of changed ploidy in sexually reproducing crops, such as wheat, are therefore exceptional, as vegetatively propagated crops domesticated either for edible vegetative tissue or for fruit, not seed crops, are most strongly associated with ploidy changes.

Reproductive strategies

Our dataset included a relatively even distribution of outcrossing, self-fertilizing and vegetatively propagated crops (Table 2). Similar proportions were also found in a review of 124 crops by Simmonds (1976; 1979). There is considerable variation in reproductive strategies for a number of crops: of the total 203 crops, both self-fertilizing and outcrossing strategies characterized 25 crops, both self-fertilizing and vegetative propagation strategies characterized 32 crops and both outcrossing and vegetative propagation strategies characterized 66 crops.

A number of crops exhibit a change in reproductive strategy between their wild and domesticated forms, either from outcrossing to self-fertilizing or from sexual reproduction to vegetative propagation. This is considered an important

feature of crop domestication because, similar to a change in ploidy level, it is a mechanism for establishing reproductive isolation, allowing farmers to maintain desired phenotypes. It also allows for the production of desirable fruits with few or no seeds (Gepts, 2004). Both of these types of change in the reproductive strategy used under cultivation are frequently documented in this dataset. These changes occurred in 27% of the crops reviewed.

Shifts from outcrossing to a self-fertilizing system are considered a relatively common domestication syndrome trait for fruit and seed crops (e.g., Gross and Olsen, 2010; Roumet et al., 2012). Overall, however, our data do not support this theory as common: results of LF analyses showed a negative association between self-fertilizing crops and changes in reproductive strategy (Appendix I, Table S6). Furthermore, this transition characterized under 20% of the self-fertilizing crops, indicating that most were already self-fertile in their wild state. This likely contributed to making them favorable candidates for domestication.

The domestication syndrome

We quantified differences in the frequencies of domestication traits characterizing different groups of crops. The most common domestication syndrome traits are changes in secondary (or specialized) metabolites (e.g., loss of bitter or toxic compounds, pigment changes), occurring in 66% of crops, followed by changes to the morphology of aerial vegetative parts, and changes to fruits (Fig. 2b; Appendix I, Table S3). Although loss of shattering is a classic domestication trait (Purugganan and Fuller, 2009; Salamini et al., 2002), it only occurred at a low frequency (16%). Beginning ca. 4000 y.a., annual domestication began to decline (Fig. 2a), and, correspondingly, changes to seed morphology

decreased in frequency and loss of shattering stabilized (Fig. 2b). Perennial crop domestication increased 2-3000 y.a. and, correspondingly, domestication traits related to aerial vegetative parts, fruit morphology, and secondary metabolites also increased sharply (Fig. 2a,b).

One caveat to discussion of these trends, however, is that our perceptions of domestication may be distorted by the plant groups and organs that are best conserved in the archaeobotanical record. Carbonized seed remains from the burning of food waste, starch analyses from early tools, and identification of phytoliths disproportionately reflect certain groups, in particular grasses, pulses and tubers (Smith, 1968). In contrast, traits shown to be prominent in recent times, such as changes to fruit morphology and changes to secondary metabolites are less easily captured in the archaeological record. This may in part account for their lower observed prevalence in earlier periods.

In our dataset, many domestication syndrome traits occurred at different frequencies in different regions (Fig. 3b). Changes in secondary metabolites, fruit, aerial vegetative parts, and seed morphology, were common across all regions. However, traits closely linked with particular plant life cycles often differed with the influence of climate and ecology on the respective regional floras. Regions with arid climates, such as the Near East and Abyssinia, are characterized by a large degree of domesticated annuals and high frequencies of seed morphology and non-shattering as domestication syndrome traits. Regions with humid climates, such as those of Near Oceania and Amazonia, do not have crops with non-shattering traits, corresponding to the lower occurrence of annuals. They also

have fewer crops with a change in life cycle compared to arid regions; this is consistent with a year-round growing season (Fig. 3).

Further differences in domestication syndrome trends can potentially be explained by harvesting techniques, technologies and preferences specific to geographical regions. For example, in South Asia, sickle harvest of Asian rice resulted in fixation of the non-shattering trait (*sh4*; Li et al., 2006; Ishikawa et al., 2010). However, in West Africa, African rice was harvested by swinging a basket; as this method favored shattering phenotypes, non-shattering was never selected for (Carney, 2001; Linares, 2002). In some cases, similar crops were domesticated for different food organs in different regions. For example, amaranth provides a grain in Mesoamerica but is exploited as a potherb in Africa (Grubben, 2004); and lettuce is used for edible leaves in the Mediterranean but was selected for an enlarged edible stem in China (Whitaker, 1969). Overall, we find that there is a high diversity of suites of domestication traits in food crops. This is contrary to the classical concept of the domestication syndrome; that there is a limited number of generally observed patterns of convergent evolution in crop plants (Hammer, 1984). Although the concept of a "syndrome" can be a useful tool for education, it can oversimplify patterns in nature. This has previously been argued in the case of the "pollination syndrome" (Ollerton et al., 2009).

Spatial and temporal trends

Archaeological evidence supports 24 separate regions where crop domestication occurred independently (Purugganan and Fuller, 2009). On the basis of floristic regions, our data supported 28 regions where crop domestication occurred (Fig. 4; Appendix I, Tables S4-S5), and 27 regions where one or more

domesticates had probable origins as exploited wild species (Appendix I, Tables S4-S5).

The patterns of domestication activity in the different centers of domestication are extremely variable over time (Fig. 4). Peaks in domestication activity in our dataset coincide with the rise of major civilizations. The highest rates of domestication in Egypt occur during the prosperous New Kingdom period of the Egyptian empire (Fig. 4g). There was a spike in domestication events in the Mediterranean during the period of the early Roman Empire which accounts for one third of the crops domesticated worldwide during that interval (Fig. 4h). The two peaks of domestication in China coincide with the spread of Chinese civilization, religion, and medicinal knowledge associated with the Yellow Emperor starting near the Yellow River (4-5000 y.a.; Fig. 4f), and the spread of the Eastern Han into the northern Mekhong river valley of Indochina (2-3000 y.a.; Fig. 4h). There is also a peak in domestication in Indochina during this interval. Relatively few crops have been domesticated in North America compared with other regions (Table 2). Although many of these (e.g., cranberry, wild rice) were used in their wild form for long periods, domestication in this region only peaked in the last 500 years, during and after the "Columbian exchange" (Gepts and Papa, 2002) (Fig. 4k). Many are the result of modern breeding programs (e.g., cranberry, highbush blueberry, pecan). This provides a contemporary example of domestication as an ongoing process driven by diffusion of technology.

The regions of Amazonia and West Africa, were strongly associated with both "underutilized" and "semi-domesticated" crops in LF results (Appendix I, Table S6). Light management and traditional harvesting of semi-domesticated

forest products in the Amazon and West Africa has meant that their production and distribution has been relatively restricted (Harlan, 1992; Clay and Clement, 1993). The low visibility of many crops domesticated in these regions could account for the relatively recent acceptance of Amazonia and West Africa as centers of domestication (Heller et al., 1997). Today, in both regions, a number of species are entering plant breeding programs with an increased focus on previously undomesticated crops in West Africa (e.g., baobab, dika; Van der Stege, 2010), and improvement upon domesticated and semi-domesticated crops in Amazonia (e.g., açaí, guarana; Brondizio, 2008; Clement, 1999a; Clement, 1999b; Clement et al., 2010).

The marginality model posits that domestication is frequently driven by the reproductive isolation between wild and domesticated forms caused by the removal of a plant from its native range (Binford, 1968; Flannery, 1969; Verhoeven, 2004). We tested this model using the data for region of origin of the ancestor and center of domestication of the crop and found that only a small proportion (12%) of crops were domesticated outside of their native range, making this model an exception rather than the rule in explaining domestication (Table 2). Many of these exceptions are recently domesticated crops such as grapefruit and kiwi, driven by contemporary movement of germplasm. Kiwi was domesticated in New Zealand although it had been exploited as a wild crop for thousands of years in China (Ferguson and Seal, 2008), and grapefruit, a hybrid of *Citrus sinensis* from South East Asia and *Citrus maxima* from Indonesia, was domesticated in Barbados in the 1820's (Kumamoto et al., 1987)./

The question of whether a crop has been domesticated once versus multiple times is frequently an ongoing debate (see Section II.6; Zohary and Spiegel-Roy, 1975; Olsen and Gross, 2008; Fuller et al., 2011). The detailed analyses needed to differentiate single from multiple origins have not been performed for many crops. Furthermore, the genetic signature of multiple origins can be obscured by historic bottlenecks, gene flow, genetic drift, and admixture (Allaby et al., 2008), and conclusions are strongly dependent on the sampling strategy and the abundance of molecular data available (e.g., Smith, 2006; This et al., 2006; Blackman et al., 2011; Molina et al., 2011). Multiple origins have been proposed for only 38 (19%) of the crops analyzed. In the grasses, however, it appears that multiple origins have occurred more frequently.

One third of the crops with proposed multiple origins originate in the Mediterranean region, a region contiguous with three other regions supporting crop domestication, including two centers of origin as defined by Vavilov (Abyssinia, Near East). This high proportion of crops with multiple origins may reflect the role of trading networks between North Africa, the Near East and Northern Europe that played an early role in the dissemination of crops and technical knowledge. One example of this is Nubian cotton (*Gossypium herbaceum* L.): the Roman textile market was purchasing cotton from India and that trade may have influenced Nubian farmers to domesticate their local cotton (Van der Veen, 2011; Palmer et al., 2012).

For the major domesticates, multiple origins were rare in Eurasia but common in the Americas. It has been proposed that ease of crop diffusion along the East-West axis of Eurasia, combined with narrow ranges of the wild ancestors,

limited multiple domestication events. In comparison, the Americas had slower crop diffusion along a North-South axis (Diamond, 2002). In our larger dataset, however, there were only slightly more crops with multiple origins in the New World (28%) compared to the Old World (22%), suggesting that the difference in axes of the continents is not a sufficient explanation for the number of crops with multiple origins. These multiple origin crops, however, have wild ancestors that occur in a significantly larger number of floristic regions than crops with single domestication events (Table 3). This supports the theory that a broad distribution of the wild ancestor is a likely factor predisposing a species to multiple domestications, although further investigation of this question is required.

Recent evidence from archaeological data, modeling, and evolutionary genetics supports a protracted transition model, which maintains that domestication occurs gradually over time at rates comparable with evolution under natural selection (Tanno and Wilcox, 2006; Purugganan and Fuller, 2009; Fuller et al., 2011; Purugganan and Fuller, 2011b). Our estimates of the time to domesticate a plant species fall in line with expectations of a protracted model (Fig. 5; Allaby et al., 2008). Furthermore, the time interval between initial exploitation of the wild ancestor and domestication decreases as the time of first use of the common ancestor progresses toward the present (Fig. 5). Although this trend is undoubtedly influenced by the greater availability of more recent archaeobotanical material and by the inherent bias in using the present day as the frame of reference, it merits further investigation. A number of factors relating to selection could accelerate the domestication rate over time; in particular, transitions from unconscious to conscious selection, increasing knowledge and

innovation in agricultural practices and technologies, and the development of modern breeding practices. In addition, stronger barriers to gene flow between wild and domesticated forms caused by increased environmental patchiness and increased long distance travel could facilitate more rapid fixation of domestication traits.

Utilization of plant parts

A large number of our food plants were originally exploited for purposes other than food or in addition to being a food. The eggplant was likely originally used as a medicine and hide-tanner (Daunay et al., 2001), and saffron was used as a body paint, dye, and perfume (Mousavi and Bathaie 2011). Wild olive trees in the Mediterranean basin were valued for their wood with a high oil content that made it resistant to decay and allowed it to burn while wet before the fruits became exploited as food (Salavert, 2008; Breton et al., 2009; Belaj et al., 2010). The toxic cyanogenic glycosides in cassava that must be removed before consumption were useful for stunning fish, and cinnamon was likely first used in embalming practices (Baumann, 1960). Others species were originally exploited for different organs than their current cultivated forms. For example, the carrot was first cultivated for seeds that were used as both a spice and a medicine (Simon, 2000).

Comparisons between early and current crop uses identified dynamic, persistent and interconnected uses. In the dataset, 62% of the crops exhibit more current uses than early uses. This may reflect the paucity of sources on early uses for many crops, but may also reflect ongoing domestication efforts to optimize utility. The largest increases were observed in the alcohol, fodder and cosmetic

use categories; each category was nearly double the early use value. Fuel or oil, poison, food, and fiber increased over time as well, but to a lesser extent. Only two categories had fewer current uses than early uses: currency and ritual use. Despite these shifts in use, a high level of continuity between the early and current uses of most crops is noticeable. This is supported by LF results (Appendix I, Table S5).

We hypothesized that shifts in the primary use organs of a crop would cause changes in other organs, and further, that there would be a correlation between some organs used for food and non-food uses of other organs. We found plants with edible seeds were positively associated with use as fiber, and crops with a domestication syndrome trait of seed retention were associated with use of leaves and use as fiber. Therefore, results suggest that over the course of domestication for non-shattering grains, non-food harvest residues were adapted for other household uses, such as fiber, and then later as fodder when animal husbandry and domestication followed cultivation of grasses in many regions (Clutton-Brock, 1989; Verhoeven, 2004; Vigne, 2011). Fiber crops showed the least difference between early and current use categories: 71% of crops with early uses for fiber are still used for fiber, suggesting that the discovery and reliance upon these useful materials (e.g., for rope) happens early in the selection process, and that this use remains important over time. Some of the earliest domesticated fiber plants such as flax, cotton, and hemp are still important in the global textile industry despite the emergence of synthetic alternatives.

The connections between food and medicine are well documented and many cultures do not distinguish explicitly between these two uses (Balick and Cox,

1996; Pieroni and Price, 2006). The prevalence of medicinal uses is strongly reflected in the crops we sampled: 69% of the food crops are currently used for medicinal purposes, and 59% were used as medicine early in their exploitation history, although this figure is certainly underrepresented due to limited information regarding early medicinal use (Table 2). A small number of crops (14%) were initially used as medicine but only later incorporated into the diet as a food; such histories illustrate the multiple values of food plants to our health aside from providing basic nutrients. These crops are predominantly spices such as annatto, bay laurel, clove, ginger, sage, and turmeric, or stimulants including Kola, guarana, and tea. There is evidence that organoleptic preference for flavoring food in different cultures was developed in conjunction with the health needs of the people (Sherman and Hash, 2001; Nabhan, 2004). In addition, the strong correlation between medicinal and ritual uses in both early and contemporary eras points to the high cultural value of medicinal foods (Appendix I, Table S6).

Conclusions

This review is an effort to consolidate, analyze and interpret available information on crop domestication in order to quantitatively understand the changing traits, uses and geographic distributions characterizing a wide range of crop plants in various stages of domestication. The patterns detailed in this review support some established models and theories, contradict others, and identify recent trends in crop domestication as well as knowledge gaps. Because these conclusions are based largely on correlations across a large dataset, our intention is primarily to identify promising directions for further research, rather than to

draw definitive conclusions regarding specific pathways and mechanisms of crop evolution. Conclusions and perspectives from this analysis include the following:

- Reliance on a small number of model crops, especially grasses, identifies trends in domestication syndrome traits that may be exceptions rather than rules. Loss of shattering, transitions from outcrossing to self-fertilizing, and ploidy changes in sexually reproducing crops are observed less frequently than expected.
- The marginality model, by which crops are domesticated after removal from their native range is not common (12%), and many of these cases are recent domesticates produced by agricultural research centers.
- Multiple origins have been proposed for only a small subset of this dataset (19%), and these events are associated with trade networks, and range of the ancestor.
- Trees were domesticated in two waves, associated with increases in ploidy and shifts to vegetative propagation strategies.
- Trees were domesticated at a slower rate, and exhibit significantly fewer domestication syndrome traits, compared to annuals.
- Domestication syndrome traits vary by center of domestication, corresponding in part with local climates and plant life cycles.
- Suites of domestication syndrome traits differ between crops with surprisingly few common patterns, perhaps due to different temporal, geographic, and evolutionary factors.
- The most common domestication syndrome trait is changes to secondary metabolites affecting flavor, pigments and toxicity.

- Centers of domestication exhibit fluctuations in domestication activity over time, often corresponding with factors such as the expansions of major civilizations and increased trade.
- Decreasing intervals between initial exploitation of the wild ancestor and the appearance of domesticated forms demonstrate an ongoing trend towards more rapid fixation of domestication traits.
- A large proportion of global food crops (69%) are currently used medicinally.
- There are major gaps in the literature for many crop species, particularly with regards to ancestors, region of origin and domestication dates.

Crop varieties and wild relatives, while increasingly threatened by progressive climate change, habitat loss and agricultural intensification, can help provide the genetic diversity necessary for adapting to future climate risk and meeting food security needs (Fowler and Mooney 1990; Jarvis *et al*., 2008). This diversity can be secured through complementary *in situ* and *ex situ* conservation strategies (Fowler and Hodgkin, 2004; Mercer and Perales, 2010; Bellon *et al*., 2011; Jensen *et al.,* 2012). Currently, many research efforts are expanding the genetic base of our major food crops by incorporating new traits from a number of sources with an emphasis on using and conserving the gene pools present in crop wild relatives (Doebley, 1992; Gepts, 1993; Haussmann *et al*., 2003; Meilleur and Hodgkin 2004; Brown and Hodgkin 2007; Sadiki *et al*., 2007).

In North America alone, an estimated 3-5000 species of wild plants were once used as food, but today 90% of the world's food needs are met by just over

100 species (Fowler and Mooney 1990; Prescott-Allen and Prescott-Allen 1990). The cultivation and commercialization of neglected and underutilized species is increasingly recognized as a viable development strategy with benefits such as managing climate risk, enhancing agrobiodiversity and improving rural livelihoods (Padulosi et al., 1999; Giuliani 2004; Williams and Haq 2002; Wil, 2008). The compilation of available information regarding crop use, domestication history, and wild relatives can help guide both *in situ* and *ex situ* conservation efforts to maintain diversity. Comprehensive knowledge of the state of agricultural biodiversity, along with the historical trends that have shaped and driven it, is critical in guiding our efforts to promote, conserve and utilize our rich heritage of global food crops.

Tables

Table 1. The 203 crop species reviewed in this study.

Family	Crop common name	Species/Species complex
Actinidiaceae	Kiwi	*Actinidia deliciosa* Chev. Liang and Ferguson
Agavaceae	Agave	*Agave tequilana* Weber
Amaranthaceae	Amaranth	*Amaranthus caudatus* L., *A. cruentus* L., *A. hypochondriacus* L.
	Beet	*Beta vulgaris* L. ssp. *vulgaris*
	Callaloo	*Amaranthus tricolor* L.
	Huauzontle	*Chenopodium berlandieri* Moq. ssp. *nuttalliae*
	Qinoa	*Chenopodium quinoa* Willd.
	Spinach	*Spinacia oleracea* L.
Anacardiaceae	Ambarella	*Spondias dulcis* Forst. syn *Spondias cytherea* Sonn.
	Cashew	*Anacardium occidentale* L.
	Jocote	*Spondias purpurea* L.
	Mango	*Mangifera indica* L.
	Peruvian Peppertree	*Schinus molle* L.
	Pistachio	*Pistacia vera* L.
Annonaceae	Biriba	*Rollinia mucosa* (Jacq.) Baill.
	Cherimoya	*Annona cherimola* Mill.
	Pawpaw	*Asimina triloba* (L.) Dunal
Apiaceae	Anise	*Pimpinella anisum* L.
	Carrot	*Daucus carota* L. ssp. *sativus*
	Celery	*Apium graveolens* L. var. *dulce* and var. *rapaceum*
	Cumin	*Cuminum cyminum* L.
	Dill	*Anethum graveolens* L.
	Parsely	*Petroselinum crispum* (Mill.) Nyman ex A.W. Hill
Araceae	Giant taro	*Alocasia macrorrhizos* (L.) G. Don
	Malanga	*Xanthosoma sagittifolium* (L) Schoot
	Taro	*Colocasia esculenta* (L.) Schott
Arecaceae	Açaí	*Euterpe oleracea* Mart.
	African oil palm	*Elaeis guineensis* Jacq.
	Areca nut	*Areca catechu* L.
	Coconut	*Cocos nucifera* L.
	Date Palm	*Phoenix dactylifera* L.

Family	Crop common name	Species/Species complex
	Peach palm	*Bactris gasipaes* Kunth ssp. *utilis* or ssp. *gasipaes*
	Sago palm	*Metroxylon sagu* Rottboell
Asteraceae	Artichoke	*Cynara cardunculus* var. *scolymus* (L.) Benth.
	Chicory	*Cichorium intybus* L.
	Endive	*Cichorium endivia* L.
	Jerusalem artichoke	*Helianthus tuberosus* L.
	Lettuce	*Lactuca sativa* L.
	Safflower	*Carthamus tinctorius* L.
	Sumpweed	*Iva annua* L. var. *macrocarpa*
	Sunflower	*Helianthus annuus* L. var. *macrocarpus* (DC.) Cockerell
Basellaceae	Ceylon spinach	*Basella alba* L.
Berberidaceae	Mayapple	*Podophyllum peltatum* L.
Betulaceae	Hazelnut	*Corylus avellana* L.
Bixaceae	Annatto	*Bixa orellana* L.
Brassicaceae	Cabbage and derivatives	*Brassica oleracea* L.
	Cress	*Lepidium sativum* L.
	Horseradish	*Armoracia rusticana* G.Gaertn., B.Mey. & Scherb.
	Maca	*Lepidium meyenii* Walp.
	Radish	*Raphanus sativus* L.
	Rapeseed (Canola)	*Brassica napus* L. var *oleifera* Delile
Bromeliaceae	Pineapple	*Ananas comosus* (L.) Merr.
Burseraceae	Pili nut	*Canarium ovatum* Engl.
Cactaceae	Dragonfruit	*Hylocereus undatus* (Haw.) Britton & Rose
	Pitaya	*Stenocereus queretaroensis* (Weber) Buxbaum
	Prickly pear	*Opuntia ficus-indica* (L.) Mill.
Cannabaceae	Hemp	*Cannabis sativa* L.
	Hops	*Humulus lupulus* L.
Capparidaceae	Caper	*Capparis spinosa* L.
Caricaceae	Papaya	*Carica papaya* L.
Celastraceae	Khat	*Catha edulis* Forsk.
Convolvulaceae	Kangkong (water spinach)	*Ipomoea aquatica* Forsk
	Sweet potato	*Ipomoea batatas* (L.) Lam.
Cucurbitaceae	Bitter melon	*Momordica charantia* L.
	Bottle gourd	*Lagenaria siceraria* (Molina) Standl.
	Cucumber	*Cucumis sativus* L.

Family	Crop common name	Species/Species complex
	Loofah	*Luffa aegyptiaca* Mill.
	Pumpkin (giant pumpkin)	*Cucurbita maxima* Duchesne
	Squash and pumpkin	*Cucurbita pepo* L.
	Watermelon	*Citrullus lanatus* (Thunb.) Matsum. & Nakai
Cyperaceae	Tiger nut	*Cyperus esculentus* L.
Dioscoreaceae	Indian yam	*Dioscorea trifida* L.
	Mountain yam (Japanese)	*Dioscorea opposita* Thunb.
	Ube/yam	*Dioscorea alata* L.
	Guinea yam (White yam)	*Dioscorea rotundata* complex: *D. rotundata* Poir. and *D. cayenensis* Lam.
Ebenaceae	Chocolate pudding fruit	*Diospyros nigra* (J.F. Gmel.) Perrier (formerly *D. digyna* Jacq.)
	Persimmon (Japanese)	*Diospyros kaki* Thunb.
Ericaceae	Blueberry (highbush)	*Vaccinium corymbosum* L.
	Cranberry	*Vaccinium macrocarpon* Ait.
Euphorbiaceae	Cassava	*Manihot esculenta* Crantz ssp. *esculenta*
Fabaceae	Carob	*Ceratonia siliqua* L.
	Chickpea	*Cicer arietinum* L.
	Common bean	*Phaseolus vulgaris* L.
	Cowpea	*Vigna unguiculata* (L.) Walp.
	Fava bean	*Vicia faba* L. var. *minor, V. faba* L. var. *major*
	Hyacinth bean	*Lablab purpureus* (L.) Sweet
	Lentil	*Lens culinaris* Medik.
	Mung bean	*Vigna radiata* (L.) R. Wilczek
	Pea	*Pisum sativum* L.
	Peanut	*Arachis hypogaea* L.
	Soy	*Glycine max* (L.) Merr.
	Tamarind	*Tamarindus indica* L.
Fagaceae	Oak	*Quercus spp.*
	Sweet chestnut	*Castanea sativa* Mill.
Ginkgoaceae	Ginkgo	*Ginkgo biloba* L.
Gnetaceae	Eru	*Gnetum africanum* Welw.
	Spanish joint fir	*Gnetum gnemon* L.
Grossulariaceae	Black currant	*Ribes nigrum* L.
Iridaceae	Saffron	*Crocus sativus* L.
Irvingiaceae	Dika	*Irvingia gabonensis* (Aubry-Lecomte

Family	Crop common name	Species/Species complex
		ex O'Rorke) Baill.
Juglandaceae	Pecan	*Carya illinoinensis* (Wangenh.) K. Koch
	Walnut	*Juglans regia* L.
Lamiaceae	Basil	*Ocimum basilicum* L.
	Lavender	*Lavandula angustifolia* Mill.
	Mint	*Mentha spicata* L.
	Sage	*Salvia officinalis* L.
Lauraceae	Avocado	*Persea americana* Mill.
	Bay laurel	*Laurus nobilis* L.
	Cinnamon	*Cinnamomum verum* J. Presl.
	Sassafras	*Sassafras albidum* (Nutt.) Nees
Lecythidaceae	Brazil Nut	*Bertholletia excelsa* Humb. & Bonpl.
Liliaceae	Asparagus	*Asparagus officinalis* L.
	Garlic	*Allium sativum* L.
	Onion	*Allium cepa* L.
Linaceae	Flax	*Linum usitatissimum* L.
Lythraceae	Pomegranate	*Punica granatum* L.
Malvaceae	Baobab	*Adansonia digitata* L.
	Cacao	*Theobroma cacao* L.
	Cola (Kola)	*Cola nitida* (P.Beauv.) Schott & Endl.
	Cotton	*Gossypium hirsutum* L.
	Durian	*Durio zibethinus* Murr.
	Okra	*Abelmoschus esculentus* (L.) Moench
	Small leaved white cross berry	*Grewia tenax* (Forssk.) Fiori
	Tossa jute	*Corchorus olitorius* L.
Moraceae	Breadfruit	*Artocarpus altilis* (Parkinson) Fosberg
	Fig	*Ficus carica* L.
	Mulberry	*Morus alba* L.
	Sycamore fig	*Ficus sycomorus* L.
Musaceae	Banana	*Musa acuminata* Colla. and *M. balbisiana* Colla.
	Enset	*Ensete ventricosum* (Welw.) Cheesman
Myrtaceae	Clove	*Syzygium aromaticum* (L.) Merr. & L.M. Perry
	Guava	*Psidium guajava* L.
	Malay apple	*Syzygium malaccense* (L.) Merr. & L.M. Perry
Oleaceae	Olive	*Olea europaea* L. ssp. *europaea* var. *europaea*
Orchidaceae	Vanilla	*Vanilla planifolia* L.
Oxalidaceae	African wood-sorrel	*Oxalis pes-caprae* L.

Family	Crop common name	Species/Species complex
	Cucumber tree	*Averrhoa bilimbi* L.
	Oca	*Oxalis tuberosa* Molina
	Starfruit	*Averrhoa carambola* L.
Pandanaceae	Pandan	*Pandanus amaryllifolius* Roxb.
Pedaliaceae	Sesame	*Sesamum indicum* L.
Pinaceae	Pinyon pine	*Pinus edulis* Engelm.
Piperaceae	Black pepper	*Piper nigrum* L.
	Hoja santa	*Piper auritum* Kunth
Poaceae	Barley	*Hordeum vulgare* L. ssp. *vulgare*
	Bread Wheat	*Triticum aestivum* L.
	Corn	*Zea mays* L. ssp. *mays*
	Millet	*Panicum miliaceum* L.
	Millet (foxtail)	*Setaria italica* (L.) P. Beauvois
	Millet (pearl)	*Pennisetum glaucum* (L.) R. Br.
	Oat	*Avena sativa* L.
	Rice (African)	*Oryza glaberrima* Steud.
	Rice (Asian)	*Oryza sativa* L.
	Rye	*Secale cereale* L.
	Sorghum	*Sorghum bicolor* (L.) Moench ssp. *bicolor*
	Sugar cane	*Saccharum officinarum* L.
	Teff	*Eragrostis tef* (Zucc.) Trotter
	Wild rice (American)	*Zizania palustris* L.
	Wild rice (Manchurian)	*Zizania latifolia* Turcz.
Polygonaceae	Buckwheat	*Fagopyrum esculentum* Moench.
Proteaceae	Macadamia	*Macadamia integrifolia* Maiden & Betche and *M. tetraphylla* L.A.S.Johnson (and hybrids of the two)
Ranunculaceae	Blackseed	*Nigella sativa* L.
Rhamnaceae	Jujube	*Ziziphus jujuba* Mill.
Rosaceae	Almond	*Prunus dulcis* (Mill.) D.A. Webb
	Apple	*Malus domestica* Borkh. (syn. *Malus pumila* Mill.)
	Apricot	*Prunus armeniaca* L.
	Cherry	*Prunus avium* L.
	Peach	*Prunus persica* Miller
	Quince	*Cydonia oblonga* Mill.
	Red raspberry (European)	*Rubus idaeus* L. ssp. *idaeus*
	Strawberry	*Fragaria x ananassa* Duchesne ex Rozier

Family	Crop common name	Species/Species complex
Rubiaceae	Coffee	*Coffea arabica* L.
	Noni	*Morinda citrifolia* L.
Rutaceae	Citron	*Citrus medica* L.
	Grapefruit	*Citrus paradisi* Macf.
	Lemon	*Citrus limon* (L.) Burns
	Mandarin	*Citrus reticulata* Blanco.
	Sichuan peppercorn	*Zanthoxylum bungeanum* Maxim.
	Sweet orange	*Citrus sinensis* (L.) Osbeck
	White sapote	*Casimiroa edulis* Llave & Lex.
Sapindaceae	Ackee	*Blighia sapida* Kon.
	Guarana	*Paullinia cupana* Kunth var. *sorbilis* (Mart.) Ducke
	Lychee	*Litchi chinensis* Sonn.
Sapotaceae	Cainito (Star Apple)	*Chrysophyllum cainito* L.
	Shea	*Vitellaria paradoxa* C. F. Gaertn.
Schisandraceae	Star anise	*Illicium verum* Hook.f.
Solanaceae	Cannibal's tomato	*Solanum viride* Sprang.
	Cayenne pepper	*Capsicum frutescens* L.
	Chili pepper	*Capsicum annuum* L. var. *annuum*
	Cocona	*Solanum sessiliflorum* Dunal
	Eggplant	*Solanum melongena* L.
	Gboma eggplant	*Solanum macrocarpon* L.
	Pepino	*Solanum muricatum* Aiton.
	Potato	*Solanum tuberosum* L.
	Scarlet eggplant	*Solanum aethiopicum* L.
	Tomato	*Solanum lycopersicum* L.
Theaceae	Tea	*Camellia sinensis* (L) O. Kuntze var. *assamica* and var. *sinensis*
Vitaceae	Grape	*Vitis vinifera* L. ssp. *vinifera*
Zamiaceae	Bread tree	*Encephalartos altensteinii* Lehm.
	Grahamstown cycad	*Encephalartos caffer* (Thunb) Lehm.
Zingiberaceae	East Indian Arrowroot	*Curcuma angustifolia* Roxb.
	Ginger	*Zingiber officinale* Roscoe
	Turmeric	*Curcuma longa* L.

Table 2. The number of crops assigned to the different categories and subcategories of information considered in this review (detailed definitions of categories and subcategories are available in Appendix I, Table S3).

Category	Subcategory		Number of crops
Classification			
	Monocot		41
	Dicot		156
	Gymnosperm		6
Lifecycle			
	Annual[1]		85
	Biennial[1]		13
	Perennial[1]		150
		Tree-like	76
		NonTree	75
Ploidy			
	Diploid[1]		165
	Polyploid[1,2]		71
		Allopolyploid	12
		Autopolyploid	24
	Unknown		6
Reproductive Strategy			
	Self-fertilizing[1]		95
	Outcrossing[1]		102
	Vegetative[1]		115
Center of Domestication			
	West Africa Delta		14
	Eastern North America		15
	Abyssinia		14
	Central America		25
	Central Andean		13
	Central Asian		9
	China		24
	Indo-Burma		27
	Mediterranean		30
	Near East		27
	Near Oceania		20
	Amazonia		15
Domestication Information			
	Domesticated[1]		160
		Multiple proposed	38
		Outside native range	23
		Autochthonous	169

Category	Subcategory		Number of crops
Domestication Information (cont.)			
		Date known or proposed	184
	Semi-domesticated[1]		37
	Undomesticated[1]		15
	Wild ancestor	Known/ proposed	158
		Earliest date known/ proposed	169
Traits of the Domestication Syndrome			
	Nonshattering		32
	Fruit characteristics		91
	Aerial (vegetative characteristics)		93
	Secondary metabolite		133
	Seed characteristics		78
	Reproductive strategy		54
	Life cycle shift		29
	Change in ploidy		37
Conservation/ Utilization			
	Model		66
	Minor		95
	Underutilized		87
	Ex situ	Global (WIEWS)	188
		GRIN	149
		CGIAR	75
Uses		**Early**	**Current**
	Currency	8	0
	Ritual/ Ornamental	81	57
	Cosmetic/ Dye	42	85
	Food	174	200
	Fodder	35	79
	Fiber	55	59
	Fuel	21	40
	Alcohol	25	59
	Medicine	121	140
	Poison	12	17

[1] These are non-exclusive categories, i.e., one crop may be assigned to more than one category.

[2] For some polyploid crops, insufficient information was available to distinguish between allopolyploidy and autopolyploidy.

Table 3. Comparison of the rate of domestication, the number of traits of the domestication syndrome, and the range of crop wild ancestors for different categories of crops, using unpaired, one-tailed, Student's t-tests. Bold P-values indicate significance at 95% confidence.

	Group A			Group B			Test result		
Variable tested	**Group definition**	**N**	**Mean (SE)**	**Group definition**	**N**	**Mean (SE)**	**t-value**	**df**	**P-value**
Time from exploitation to domestication	Trees	39	3767 (467)	Annuals	32	2638 (305)	-2.02	63	**0.0235**
Time from exploitation to domestication	Vegetative root plus perennial fruit crops	77	3147 (318)	Annual seed crops	43	2424 (322)	1.48	118	0.1400
Number of traits of the domestication syndrome	Trees	76	2.54 (0.17)	Annuals	85	3.41 (0.17)	3.54	158	**0.0003**
Number of traits of the domestication syndrome	Perennial fruit crops	81	3.13 (0.16)	Annual seed crops	49	3.60 (0.22)	-1.92	128	**0.0286**
Number of traits of the domestication syndrome	Vegetative root crops	23	3.00 (0.34)	Annual seed crops	49	3.60 (0.22)	-1.32	70	0.0873

	Group A			Group B			Test result		
Variable tested	**Group definition**	**N**	**Mean (SE)**	**Group definition**	**N**	**Mean (SE)**	**t-value**	**df**	**P-value**
Number of floristic regions where the wild ancestor occurs	Crops with single domestication events	150	1.66 (0.08)	Crops with multiple domestication events	38	2.29 (0.21)	-3.36	186	**0.0009**

Figures

Figure 1. The domestication history of barley (*Hordeum vulgare* ssp. *vulgare*) from its wild ancestor (*Hordeum vulgare* ssp. *spontaneum*), shown as a continuous process along a timeline. There is a long period of initial use and exploitation of the wild ancestor during the pre-domestication period, followed by the fixation of key domestication syndrome traits such as larger grains and non-shattering and finally the development of resistant and dwarf varieties in the past century as examples of ongoing domestication. There is an approximate 4000-year time interval between the detection of hull-less types in Iran and the diffusion of this phenotype throughout Europe and Scandinavia. Information regarding the phenotypes of domesticates found in different locations are presented above the timeline and the corresponding traits and genes that are modified to produce these phenotypes are presented below the timeline. At least two independent domestication events are posited for barley, based on genetic evidence. Such a thorough understanding of domestication processes is established for very few crops, usually due to limits in the available evidence in the archaeobotanical record. (ya=yr ago)

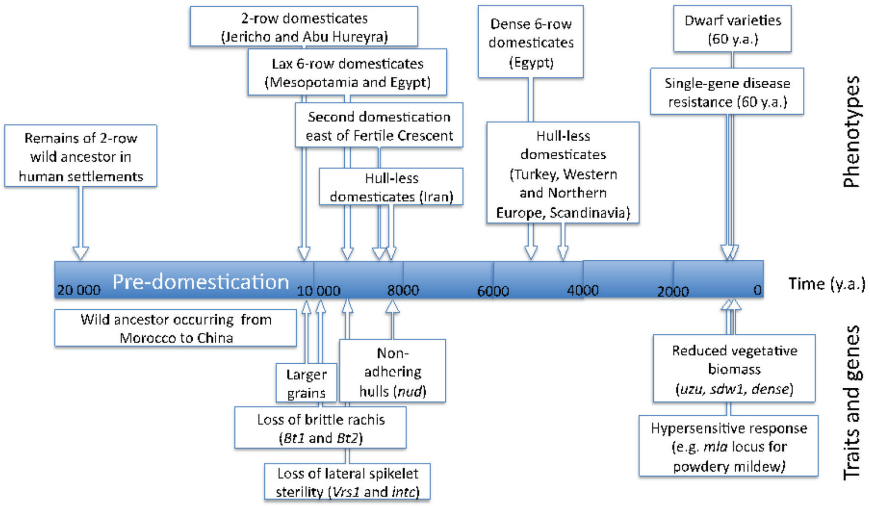
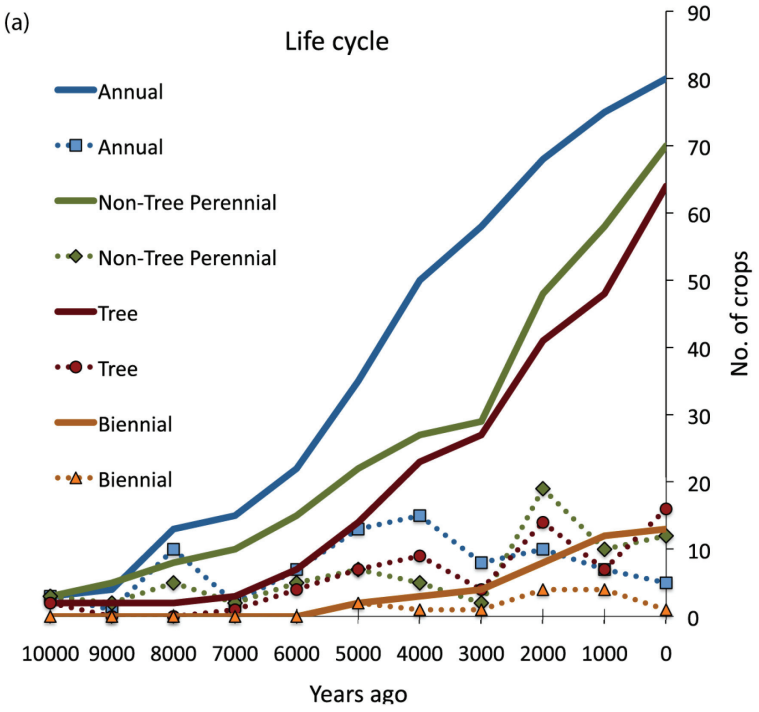

2-row domesticates (Jericho and Abu Hureyra)
Lax 6-row domesticates (Mesopotamia and Egypt)
Second domestication east of Fertile Crescent
Hull-less domesticates (Iran)
Dense 6-row domesticates (Egypt)
Hull-less domesticates (Turkey, Western and Northern Europe, Scandinavia)
Dwarf varieties (60 y.a.)
Single-gene disease resistance (60 y.a.)
Remains of 2-row wild ancestor in human settlements
Phenotypes
20 000
Pre-domestication
10 000
8000
6000
4000
2000
0
Time (y.a.)
Wild ancestor occurring from Morocco to China
Larger grains
Non-adhering hulls (nud)
Loss of brittle rachis (Bt1 and Bt2)
Loss of lateral spikelet sterility (Vrs1 and intc)
Reduced vegetative biomass (uzu, sdw1, dense)
Hypersensitive response (e.g. mla locus for powdery mildew)
Traits and genes

Figure 2. The number of occurrence of life cycle types and domestication syndrome traits for 203 food crops as a function of the time at which domestication occurred. (a) The number of occurrences of different life cycles are shown both cumulatively (solid lines) and by interval within each 1000-year period (dashed lines), from the earliest domestication events until the present. Temporal trends in the distribution of the different life cycles show that the appearance of domesticated annuals increases from 9000 to 4000 years ago, and then starts to slow. The broad dissemination and adoption of early domesticated grains such as barley, corn and wheat may have relaxed selection intensities on local cereals. An increase in the cultivation and domestication of trees and non-tree perennials coincides with the decline in addition of new annuals in the last 3000 years, and the two periods of sharp increase 6000 and 3000 years ago coincide with innovations such as vegetative propagation through cuttings and later scion grafting. The first domesticated biennials appear in our dataset only in the last 5-6000 years, and their rise between 1-3000 years ago corresponds with the peak of trade and activity of the Roman empire throughout the Mediterranean, where many biennials were domesticated. (b) The occurrence of domestication syndrome traits in crops domesticated in different 1000-year time intervals. Domestication traits involving a change in plant secondary metabolites are the most common in every 1000-year time period of the last 7000 years; the occurrence of some traits (e.g., changes in subterranean organs; life cycle; non-shattering types) have slowed or leveled off, most notably the trait for non-shattering, whose leveling off corresponds with a decline in the rate of addition of new annuals beginning 4000 years ago.


(a)
Life cycle
Annual
Annual
Non-Tree Perennial
Non-Tree Perennial
Tree
Tree
Biennial
Biennial
No. of crops
Years ago
10000 9000 8000 7000 6000 5000 4000 3000 2000 1000 0
0 10 20 30 40 50 60 70 80 90

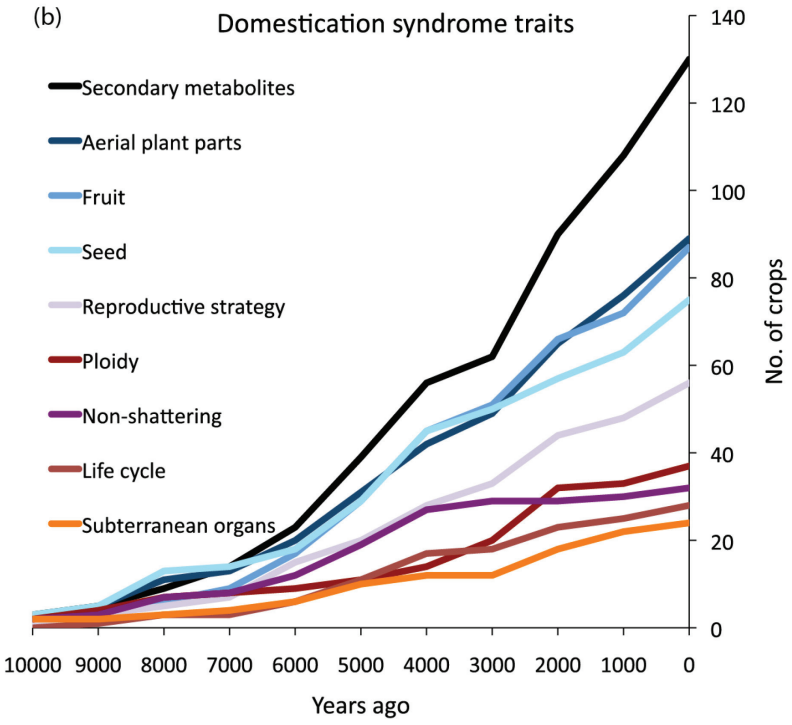
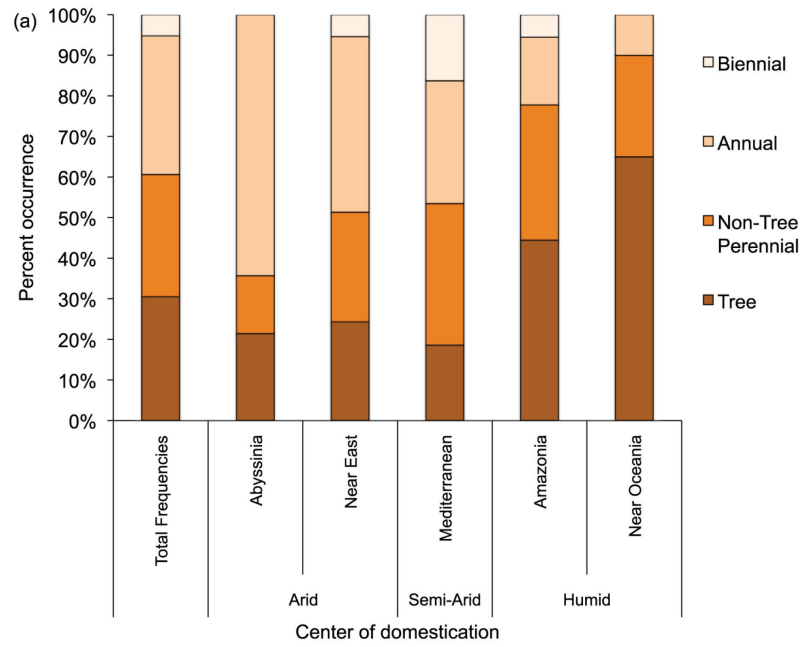

(b)
Domestication syndrome traits
Secondary metabolites
Aerial plant parts
Fruit
Seed
Reproductive strategy
Ploidy
Non-shattering
Life cycle
Subterranean organs
No. of crops
Years ago
10000 9000 8000 7000 6000 5000 4000 3000 2000 1000 0
0 20 40 60 80 100 120 140

Figure 3. The distribution of crop life cycles and domestication syndrome traits among different regions, plotted as the percent frequency of occurrence in the domesticated crops for each region. Selected regions are grouped by distinct climatic zones: arid, semi-arid, and humid. (a) The distribution of annual, biennial, non-tree and tree perennials in domesticated crops of different regions. Annuals are most prevalent in regions of arid climate, biennials are most prevalent in semi-arid climates, non-tree perennials exhibit relatively consistent proportions, and trees are most prevalent in humid climates. These results are consistent with expected proportions of such life cycles in the regional flora. (b) The distribution of domestication syndrome traits according to selected centers of domestication. Not all traits are found in the crops of all regions. No changes in seed shattering were observed in crops from Near Oceania. No changes to subterranean structures such as roots were observed in crops from Abyssinia (contemporary Ethiopia). Differences between different climates were identified: arid regions featured a smaller proportion of changes to fruits and reproductive strategy, and a greater proportion of the trait affecting seed morphology, consistent with the larger frequency of annual domestication (shown in 3a). Changes in secondary metabolites are the most common domestication trait observed in all regions.


(a)
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Percent occurrence
Biennial
Annual
Non-Tree Perennial
Tree
Total Frequencies
Abyssinia
Near East
Mediterranean
Amazonia
Near Oceania
Arid
Semi-Arid
Humid
Center of domestication

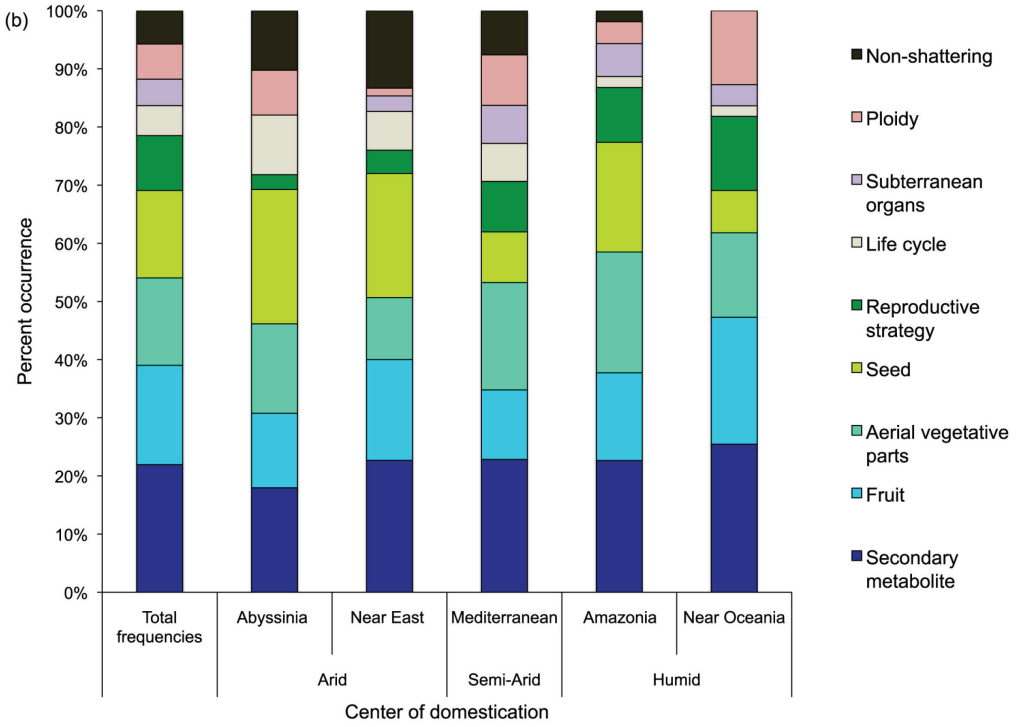
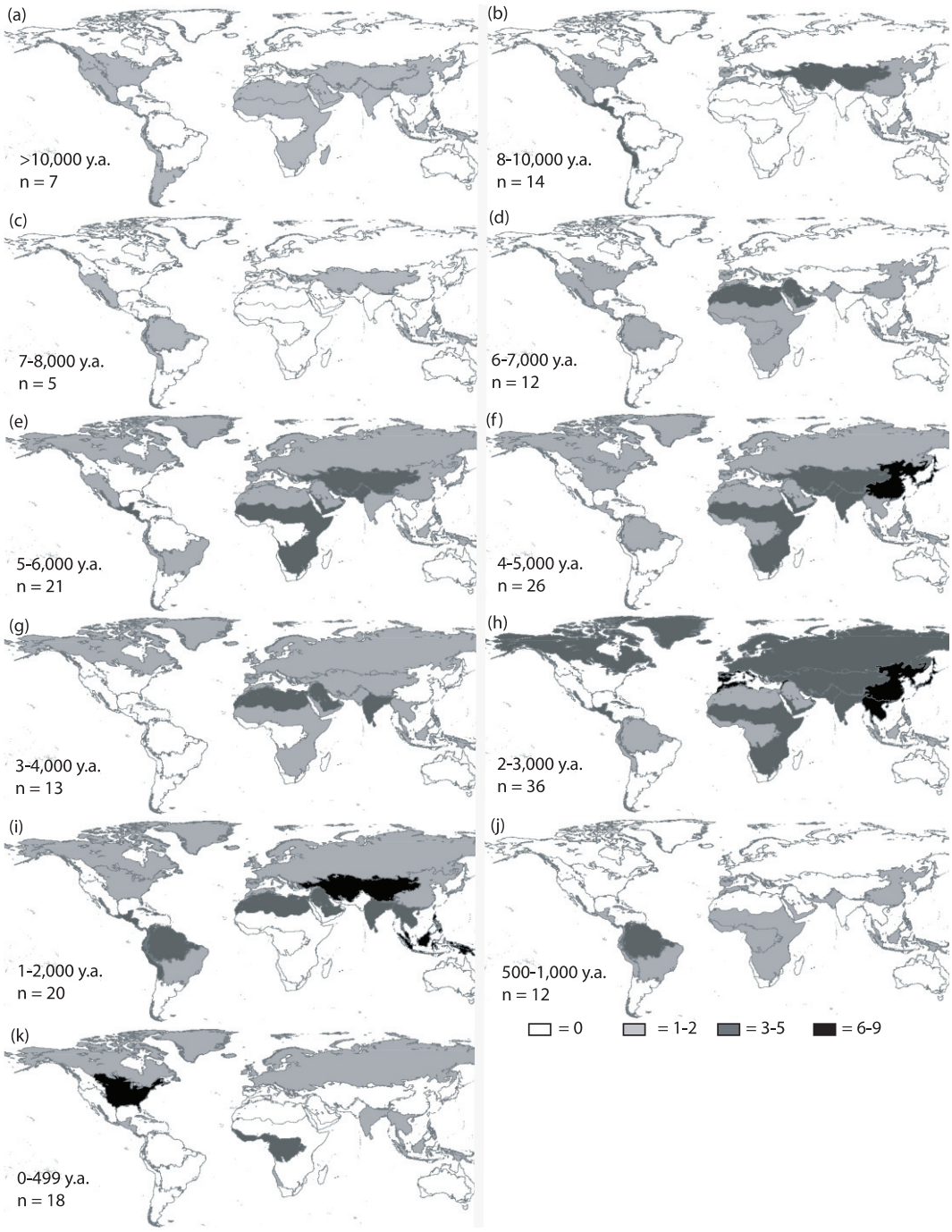

(b)
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Percent occurrence
Non-shattering
Ploidy
Subterranean organs
Life cycle
Reproductive strategy
Seed
Aerial vegetative parts
Fruit
Secondary metabolite
Total frequencies
Abyssinia
Near East
Mediterranean
Amazonia
Near Oceania
Arid
Semi-Arid
Humid
Center of domestication

Figure 4. The geographical locations of new crops domesticated worldwide represented grouped in 2000-year time intervals from >10,000 y.a. to the present (n=total number of crops). The map is subdivided according to Takhtajan's floristic regions and reflects contributions from the different centers of domestication. Shading of regions represents the number of crops domesticated in that region in each time period. Higher numbers of domestication events (represented by darker shading) often correspond with the peaks of major civilizations throughout history, such as the Yellow Emperor period in China (f); the New Kingdom of the Egyptian Empire (g); the spread of the Eastern Han from China to Indochina (h); and the early Roman Empire (h). The last 1000 years are broken into two five hundred year intervals (j,k) and reflect the influence of the Columbian exchange and contemporary breeding efforts, especially in North America (k). Near Oceania is active in domestication during every time interval (a-k); Mexico was an important site from over 10,000 y.a. to 5000 y.a. (a-e) but had no subsequent domestications in our dataset (f-k). More recently recognized centers of domestication such as Amazonia and West Africa have numerous low intensity periods of domestication.


(a)
>10,000 y.a.
n = 7
(b)
8-10,000 y.a.
n = 14
(c)
7-8,000 y.a.
n = 5
(d)
6-7,000 y.a.
n = 12
(e)
5-6,000 y.a.
n = 21
(f)
4-5,000 y.a.
n = 26
(g)
3-4,000 y.a.
n = 13
(h)
2-3,000 y.a.
n = 36
(i)
1-2,000 y.a.
n = 20
(j)
500-1,000 y.a.
n = 12
(k)
0-499 y.a.
n = 18
= 0
= 1-2
= 3-5
= 6-9

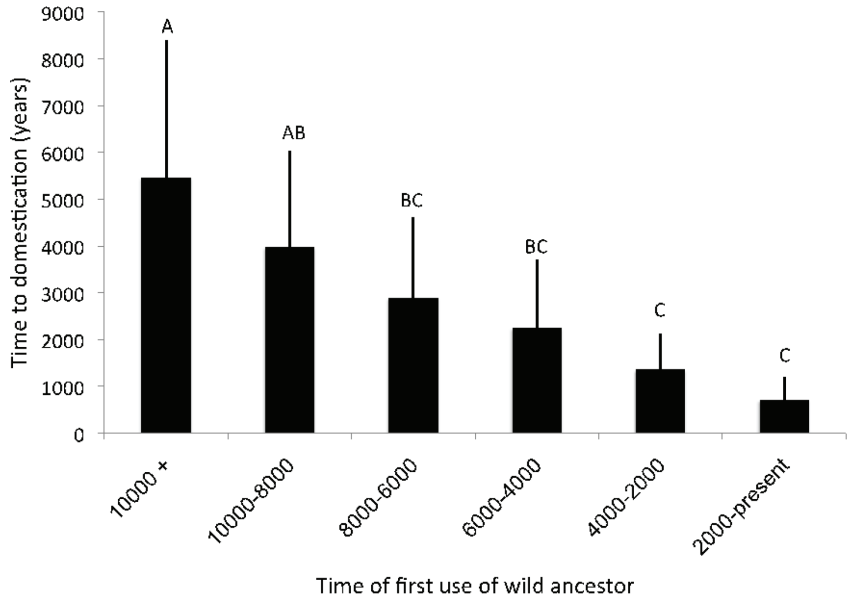


Figure 5. Mean time to domestication (+ standard error) for crops, as a function of the 2000-year time period when the first use of the crop wild ancestor was recorded. Sample sizes for the different periods are, from left to right: n = 40, n = 16, n = 15, n = 32, n = 23, n = 10. Letters denote significant differences between 2000-year periods based on results of a one-way ANOVA ($F(5,138) = 17.79$, $p<<0.0001$) and pairwise comparisons using Tukey's HSD. There is a clear, progressive decrease in mean time to domestication, in particular between crops domesticated 8000 or more years ago and more recent crops, indicating more rapid fixation of domestication traits, even though not all 2000-year time periods are significantly different from one another.

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Linking statement

In Chapter 1, I documented domestication processes in a broad sample of food crops. I demonstrated that domestication of most crops has been a protracted process. Changes in selection pressure from both human populations and the natural environment contribute to a series of micro-evolutionary changes in crops that occur after the initial transition from wild species to crop species. This supports the view that domestication is both dynamic and ongoing. In the case of barley (Figure 1 in chapter 1), documented changes in traits have occurred over at least a 10 000 year period and continue to occur today. These changes may arise through efforts by the formal breeding sector to incorporate desirable traits such as reduced stature, increased allocation to grain, and increased disease resistance. However, the cultivation of traditional varieties ("landraces"), by farmers who maintain seed through local seed exchange networks is also an important factor in exerting selection pressure on crop traits. In Chapter 1, I also note that the uses people make of crops has changed over time, and these changes are associated with phenotypic changes in the crops. For the remainder of the thesis, I focus on one specific domesticated crop, barley (*Hordeum vulgare* ssp. *vulgare*), and the relationships between populations of traditional varieties of barley in Northern Morocco, the farmers who maintain them, and the evolution of disease resistance to a co-occurring pathogen, powdery mildew (*Blumeria graminis* f.sp. *hordei*) in these populations. Gene flow among populations is an important factor in determining the potential for evolution and local adaptation of a crop species. In the case of an inbreeding crop with large seeds, such as barley, gene flow is almost exclusively mediated by humans, via seed exchange networks. The uses

and valuation that farmers attribute to a crop are a major determinant of the selection pressures that will be exerted on the crop. In chapter 2, I characterise the uses and valuation of the barley crop by people in Northern Morocco, which will influence the selection pressures on the barley crop in this region. I also assess the structure of the seed exchange network for barley in Northern Morocco.

CHAPTER 2: A case study of seed exchange networks and gene flow for barley (*Hordeum vulgare* ssp. *vulgare*) in Morocco.

Jensen HR, Belqadi L, De S, Paola, Sadiki M, Jarvis DI, Schoen DJ 2012. A case study of seed exchange networks and gene flow for barley (*Hordeum vulgare* ssp. v*ulgare*) in Morocco. *Genetic Resources and Crop Evolution*, doi.org/10.1007/s10722-012-9909-4.

Abstract

Local patterns of seed regeneration and trade that occur outside the formal breeding sector (seed exchange networks) can have a strong influence on the genetic diversity and evolution of traditional crop varieties. Despite this, little is known about the extent to which seed exchange networks influence gene flow and genetic structure in traditional crop varieties. Here we study barley (*Hordeum vulgare* ssp. *vulgare*) in rural communes of Northern Morocco in 2008 and 2009. We quantified seed regeneration and exchange by farmers within the seed exchange network using structured interviews. Using SSR markers, we also quantified the neutral genetic diversity and structure of a complex of traditional varieties referred to as *Beldi* that is managed in this exchange network. The majority of farmers (>88%) report cultivating *Beldi*. Most seeds of *Beldi* (70-90%) are maintained on-farm, while the remainder of seeds are obtained from local markets within the commune. *Beldi* has high genetic diversity and there is weak but significant genetic structure between communes (F_{ST}=0.031). From SSR marker data there is evidence of a high level of gene flow between communes not reported in interviews. Seeds purchased in local markets likely represent seeds from a larger geographic region, leading to lower genetic structure among communes than expected based on the reported level of on-farm seed regeneration and local sourcing of seed. We discuss the implications of this seed exchange network for the conservation of traditional barley varieties in the study region.

Introduction

Gene flow in plant populations occurs via both seed and pollen dispersal. In wild plant populations, geographic isolation frequently acts as a limit to gene flow and leads to the establishment of genetic structure, often in a pattern of isolation by distance (Wright, 1943). In the case of cultivated crop plants, however, gene flow by seed dispersal is usually dependent upon human intervention. For many modern crop varieties, seed dispersal occurs primarily through the formal breeding sector, with genetically homogeneous varieties distributed on a continental or intercontinental scale and cultivated in large-scale monocultures. In large areas of the developing world, however, agroecosystems devoted mainly to traditional crop varieties (often referred to as "landraces") are the principal drivers of agricultural production, representing over 10 million ha worldwide (Altieri, 2004). Farmers with small land holdings and/or agriculturally marginal lands are particularly dependent on traditional varieties that are adapted to local conditions (e.g., low rainfall, soil composition, etc.). These traditional varieties can be considered to be "population-varieties" (Bustarret, 1944) because they are genetically heterogeneous and continue to be subjected to the evolutionary forces of mutation, recombination, selection (both natural and artificial), and drift under cultivation. They typically have higher genetic diversity than formal breeding sector varieties and represent important reservoirs of potentially adaptive genes for agriculture.

In many developing countries, farmers maintain traditional varieties independently of the formal breeding sector, with seed often obtained from relatives, neighbours or local markets (McGuire, 2008). These informal seed

supply systems have been referred to using a variety of terms, including “seed exchange networks”, “farmer seed systems”, “traditional seed systems”, and “informal seed systems”. Here, we will use the term “seed exchange networks”, because it most precisely describes the complex movements of seeds among people and does not make assumptions about the social context in which seed movement is occurring or the identity of the participants in the network. Seed exchange networks are the most important factor in the movement and use of crop germplasm for traditional varieties and they therefore have a strong influence on the genetic diversity and structure of cultivated crops (Hodgkin et al., 2007). As a result, the way that seed is handled and distributed in seed exchange networks has the potential to affect the evolution of traditional crop varieties because it influences processes of gene flow, genetic drift, founder effects, local extinctions, and selection (Almekinders et al., 1994; Brush, 1995). This is particularly true for self-fertilizing crops because gene flow via pollen dispersal is restricted (Loveless and Hamrick, 1984; Brown and Brush, 2000; Epperson, 2007; Soleri and Cleveland, 2007).

A seed exchange network is structured by many cultural, economical, political, social and geographic factors, all of which may limit or enhance germplasm movement among farmers. It is therefore rare for populations of traditional crop varieties to be structured following a simple pattern of isolation by distance (Pusadee et al., 2009). Instead, they are structured according to interactions among many factors, including agro-climatic zone, elevation, biotic stressors, ethnolinguistic regions, access to markets and agricultural extension services, and marriage networks (Teshome et al., 2001; Mercer et al., 2008;

Samberg et al., 2010; Delêtre et al., 2011). If we want to implement effective conservation strategies for a crop in a particular region, it is therefore important to understand both the structure of the seed exchange network and the influence it exerts on the genetic structure of a crop.

Traditional crop varieties have previously been characterized using a number of different methods that can be broadly grouped into two categories: (1) descriptions of seed exchange networks derived from interviews with farmers and, (2) measurements of the genotypic composition and structure of a population made using genotypic markers (Soleri and Cleveland, 2001; Pautasso et al., 2012). Studies that quantify seed exchange networks with data gathered from farmer interviews have used such information to infer the likely impact that farmer seed management has on genetic diversity and structure (e.g., Alvarez et al., 2005). The flow of seeds between farmers varies with the crop and the agroecosystem, and ranges from as low as 10% of seeds or less being sourced off-farm via seed exchange networks for maize in Mexico (Badstue et al., 2007), to as high as 76% of seeds for rice in the Philippines (Carpenter, 2005). It is not generally known, however, if these levels of seed exchange translate to genetic differentiation among populations. Conversely, analysis of genotypes at neutral molecular markers such as simple sequence repeats (SSRs) can provide the best indicators of realized gene flow among populations; yet on the other hand provide no direct information about the seed exchange network that is mediating this gene flow (Parzies et al., 2004).

Although these two approaches are frequently used separately, in a recent review of seed exchange networks, Thomas et al. (2011) concluded that an

integration of genetic and ethnobotanical analyses are the key to a quantitative interpretation of how farmers' practices affect genetic diversity. The few existing studies that have concurrently examined both the seed exchange network and the genetic diversity and structure of traditional crop varieties as assessed with molecular marker data confirm this, and provide useful interpretations of the distribution of genetic diversity in the context of a seed exchange network (Perales et al., 2005; Pandey et al., 2011; Bajracharya et al., 2012; Thomas et al., 2012).

In this study, we focus on a seed exchange network for barley (*Hordeum vulgare* L. ssp. *vulgare*) in rural communes of Taounate province in Northern Morocco. In Africa, seed exchange networks are often the major sources of seeds for a community (rather than seeds from the formal sector) with as many as 20-30% of non-commercial seeds sourced off-farm via seed exchange networks (Tripp and Rohrbach, 2001). If this level of off-farm seed sourcing holds true for barley in Morocco, it would suggest a relatively high level of gene flow within the seed exchange network. However, despite the likely importance of the seed exchange network in determining gene flow, few published reports are available detailing the patterns of barley seed exchange for farmers in Morocco, and none of these have used molecular marker data (Mahdi, 2002; Rhrib et al., 2002). We used interviews with farmers to identify the varieties of barley cultivated in the study region. Results of interviews revealed that a complex of morphologically similar varieties referred to by farmers as *Beldi* dominates barley cultivation in the region. We then characterized the seed exchange network for *Beldi* by identifying and quantifying sources of seed. In conjunction, we characterized the genetic

diversity, population structure, and gene flow among populations of *Beldi* in these communes using SSR markers. Using these tools, we were able to compare and contrast expectations for genetic diversity based on farmer interviews with observed patterns of molecular diversity and thereby obtain a more comprehensive description of gene flow via the seed exchange network. Detailed knowledge of the seed system and associated genetic structure and gene flow can provide a more reliable assessment of the current state of genetic resources for this crop in the region studied. Furthermore, it can help inform the design of effective strategies for both *in situ* and *ex situ* conservation.

Materials and Methods

Study location

Although Morocco is not a center of origin for barley (Morrell and Clegg, 2007), it is considered a center of diversity (Perrino et al., 1986; Dakir et al., 2002), and 85% of the cultivated barley area is still composed of traditional varieties (Saidi et al., 2006). This proportion increases to over 95% in semi-arid and marginal sites (Jarvis et al., 2008). Traditional barley varieties in Morocco are distinct from Middle Eastern varieties (Orabi et al., 2009). Most cereal seed cultivated in Morocco is sourced from seed exchange networks, which include seed maintained on farm and seed obtained through exchanges and local markets, (89% of seed), rather than from certified seed sources (11% of seed) (Van Mele et al., 2011).

This study was conducted in the Province of Taounate, in the Rif Mountains region of Northern Morocco. This region has a mountainous

topography, with severe erosion due to deforestation, overgrazing, and cultivation on steep slopes (Sabir et al., 2002). Precipitation in this region varies between 400-600 mm a year. The region is vulnerable to drought, and a severe drought in 2007, the year prior to our study, resulted in a reduced barley harvest with yields decreasing by over 50% compared to other years (FAOSTAT, 2010). In the years of our study (2008 and 2009), the barley harvest was either average (2008), or exceptional (2009) (FAOSTAT, 2010). We selected two rural communes for study in 2008: Tissa and Ourtzagh (Fig. 1). For the household surveys in 2009 we added a third commune: Galaz (Fig. 1). Rural communes are administrative divisions that encompass one municipal center and a number of smaller villages. Each commune has a market and an agricultural extension office. Although the communes we studied are situated within the same province, they provide contrasting agro-ecological conditions representative of the region. Tissa commune, in particular, has more farms in more agriculturally productive lowland areas. Ourtzagh and Galaz communes, in contrast, have more farms at higher elevations, on marginal and sloping terrain. As a result, the study of these contrasting communes should provide a good approximation of diversity patterns and gene flow within the larger region across this agroecological gradient.

On-farm interviews and seed collection: 2008

In February of 2008 we met with farmers in the rural communes of Ourtzagh and Tissa to conduct an initial series of interviews and collect samples of the barley varieties cultivated in the region. We visited 10 villages (*douars*) in

Ourtzagh and 9 villages in Tissa (Table 1). These villages were selected to cover a broad area within each commune. Each commune has a weekly local market that is the principal location for seed exchange (Arbaoui, 2003). Villages were located at distances ranging between 0.1 Km and 14.6 Km from the market associated with their commune. We interviewed a total of 70 farmers (43 in Ourtzagh, 27 in Tissa). Farmers were randomly selected for interviews that started with an informal discussion, followed by a short, structured, interview (i.e., the interview uses a questionnaire where respondents chose between a selection of answers). Farmers were asked to identify the names of the barley varieties cultivated locally as well as their origin (traditional versus modern). These data were used to determine the overall frequency of use of the different traditional and modern varieties used in the region. During interviews, it became apparent that a traditional variety complex referred to as *Beldi* dominates barley cultivation in the region. Subsequent questions asked farmers their reasons for preferring to use *Beldi* compared to other varieties and to identify the sources of the seeds used for planting (e.g., local market, maintained on farm). We also asked farmers to specify the uses made of the barley harvest (e.g., animal fodder, human consumption, etc.).

Following the interview, a small seed sample of approximately 50 g of the variety currently under cultivation was requested from the farmer for further study using SSR markers. Geographic coordinates of each site were recorded using a GPS unit and used to calculate the Euclidean distance between the village and the local market serving the commune.

On-farm interviews: 2009

In March and April of 2009 we conducted more detailed structured individual interviews in the communes of Tissa, Ourtzagh and Galaz to obtain more detailed information regarding barley cultivation and the use and turnover of varieties in the region. A large portion of the questionnaire used in the interviews focused on *Beldi,* specifically, farmer use and valuation of *Beldi*, temporal patterns of seed regeneration and sources of gene flow within and among populations *Beldi*. We revised the questionnaires twice, using test subjects to ensure that the questions were effective for gathering the desired data and to eliminate potential ambiguities. Prior to conducting the interviews, a five-day training course in participatory rural assessment methods was organised for farmers and community members, with an equal representation of men and women among the course attendees. In addition to providing training, this course fostered the establishment of social relationships and trust among the participants. All the interviewers were selected from the course attendees.

A total of 158 farmers, representing 158 households, were randomly selected for participation in the interviews in the three rural communes: Galaz (N= 63), Ourtzagh (N=44) and Tissa (N=51). Interviews were conducted during March and April of 2009 as part of an overall assessment of farmer knowledge and perception of genetic diversity, disease incidence and pest resistance in barley and fava bean crops conducted in collaboration with IAV Hassan II and Bioversity International (Rome, Italy). Informed consent was received from all interview subjects.

Farmers were asked to report the area and number of plots devoted to barley cultivation on their farms. They also reported the different varieties they were cultivating and the area devoted to each variety was then used to calculate Simpson's diversity index. We assessed change and turnover in barley varieties over time by asking farmers to name the varieties that they currently cultivate and the varieties that they have previously cultivated.

All subsequent interview questions were used for characterising the seed exchange network and, because *Beldi* dominated the cultivation area, we asked farmers to only report these data for *Beldi*. Farmers reported how long they had been cultivating *Beldi,* either as a number of years or as the statement "for a long time". To determine how often new *Beldi* seeds are introduced to the *Beldi* seed stock maintained on-farm, farmers were asked to report the number of years between seed changes (i.e., times when they procure seed from an outside source, such as a market). Some farmers answered, "it depends", and specified that renewal is dependent on variable external factors (e.g., drought, seed loss during storage), resulting in irregular intervals of seed renewal. Farmers were also asked to report the reason underlying their decision to procure seeds from an outside source. Farmers then reported the volume of seed obtained from each of several potential different sources. This included seed that was maintained on-farm, obtained from neighbours, local markets, and seed companies. The mean percentage of seed obtained from each source was then calculated for each commune.

Genetic diversity analysis

To genotype barley samples, we tested a subset *of Beldi* seeds collected from farmers during the 2008 interviews. Seeds came from four randomly selected villages in each commune and from two to four farmers within each village. For each sample, three to four seeds were used. This resulted in a total of 84 samples (37 in Tissa, 47 in Ourtzagh) (Table 1). Seeds were germinated and DNA was extracted from a 3 cm segment of the primary leaf using the QIAGEN Dneasy plant mini kit (QIAGEN). DNA samples were genotyped using single sequence repeat (SSR) markers at six loci located on separate chromosomes. The selected loci were: Bmag0013, Bmag0321, Bmag0345, Bmac0316, Bmag0125 and Ebmac0541 (Ramsay et al., 2000). Each locus was amplified using sequence-specific primers tagged with an M13 tail and a fluorescently labelled M13 primer (Schuelke, 2000). Alleles were resolved on an acrylamide gel using a Li-Cor sequencer (Model 4300) and allele size was quantified using SAGA software (Li-Cor). Detailed methods have previously been described elsewhere (Jensen et al., 2012).

We characterized genetic diversity parameters including allelic richness, and allelic diversity per locus and per site (commune) using the program FSTAT for windows, V 2.9.3.2 (Goudet, 2002). Because sample sizes were slightly different between communes, we also performed rarefaction analysis to calculate adjusted allelic richness (standardized to a common sample size) (Kalinowski, 2004). For this, we used the program HP-RARE V.1.1 (Kalinowski, 2005) and set the number of genes per site to 30, as this was a number slightly lower than that of the site with the lowest number of genes. We also calculated private allelic

richness adjusted for population size, as an indicator of the genetic uniqueness of the barley populations in the two communes.

To compare the partitioning of genetic variation among villages and between communes we conducted an analysis of molecular variance (AMOVA) using Arlequin 3.5 (Excoffier and Lischer, 2010). To detect both fine-scale local structure and slightly broader structure we calculated pairwise F_{ST}-values among villages and between communes using the program MSA (Daniel and Christian, 2003). We calculated the number of migrants per generation between the two communes from the values of the fixation index (F_{ST}) using the equation, $F_{ST} = 1/(1+4N_em)$, where N_em represents the absolute number of migrant organisms entering the population each generation (Wright, 1943). This method provides an estimate for migration but makes assumptions regarding population parameters that may be violated in real populations. Because barley is inbreeding, random mating and Hardy-Weinberg equilibrium within subpopulations are not expected. Moreover, many generations are required to reach genetic equilibrium between drift and migration, and it is unlikely that it has been established in a crop that is subject to changes in population structure due to human-mediated seed movement.

Because of these potential problems in the calculation of migration rates using F_{ST}-based measures, we also used two specialized programs, Ima2 (Hey and Nielsen, 2007) and MIGRATE (Beerli and Felsenstein, 2001; Beerli, 2006), that use the coalescent theory and Bayesian methods to calculate long-term migration rates between populations. Ima2 uses a model that separates population isolation from migration (gene flow), and is therefore particularly appropriate to our data.

Furthermore, Ima2 does not assume genetic equilibrium and is appropriate for populations that may have recently separated. MIGRATE does not make this distinction, but is still a useful estimator for long-term gene flow. We calculated population migration rates ($M=2N_em$) between communes and effective population sizes scaled for a diploid organism ($\theta=4N_e\mu$) within each commune using both programs.

In Ima2, we ran a Bayesisan inference with uniform priors for migration rate, theta, and splitting time of the population. Prior values were determined empirically based on outputs of initial runs. A stepwise mutation model was applied. We used a burn-in of 1000, 20 heated chains with a geometric heating scheme, and swap rates between 40% and 80% between successive chains. A total of 100 000 samples were collected. Chain mixing and convergence were verified by running successive chains from different random starting points and plotting values of estimated parameters over time.

In MIGRATE, we used the Bayesian inference option with F_{ST}-values as starting values for M and Theta and wide uniform priors for both M and Theta. We used the Brownian approximation of the ladder model to account for stepwise mutation in microsatellite markers. The range of priors was determined by a number of preliminary runs to test chain convergence and the range of possible solutions. For the final analysis we ran ten replicates of a long chain with a burn-in of 10 000, sampling increments of 20, and a total of 50 000 genealogies sampled. Slice sampling was used to determine new possible states. Four heated chains were run to explore the parameter space more thoroughly.

To determine the number of genotypic clusters present in *Beldi* in the region and assess if these clusters were associated with specific geographic locations (i.e., communes), we analyzed the data using SRUCTURE V. 2.2 (Pritchard et al., 2000). We set the number of clusters as ranging from K=1 through K=10, with five iterations for each value of K, and a burn-in length and run length of 100 000 each. The most probable number of clusters present in the data was estimated from the probability of the data for each value of K and also by using an *ad hoc* estimation method (Evanno et al., 2005). Outputs of STRUCTURE analysis were plotted using DISTRUCT (Rosenberg, 2004).

Results

On-farm interviews, 2008

During informal discussion in 2008, farmers identified the two major varieties cultivated in the region as *Beldi* and *Roumi.* Both are six-row types, but *Beldi* is considered by farmers to be the traditional local variety. In Arabic, *Beldi* translates as "local" or "of the land", while *Roumi* is considered to be an imported variety derived from the formal breeding sector. In contrast to *Beldi*, the word *Roumi* translates as "foreign" or "exotic", possibly deriving from the word "European", or "Roman". The simple distinction between the names *Beldi* and *Roumi* may, however, hide a more complex reality. Because *Beldi* means "local', the term could potentially refer to a number of morphologically distinct varieties, rather than one specific variety, particularly across a broad geographic area. In the case of our study, farmers identified a suite of morphological traits that allow them to distinguish *Beldi* from other varieties*,* including 6-row spikes, seed morphology, low spike density, a high proportion of vegetative biomass, and tall

stature. There remains the possibility, however, that *Beldi* also encompasses other morphological types not identified in this study. Furthermore, farmers sometimes conceptualize *Beldi* as a series of distinct local populations, in which case the name of an individual farmer is used to qualify a particular sample of *Beldi.* For these reasons, we have referred to *Beldi* throughout this paper as a "complex of traditional varieties", rather than as a single variety. All the other varieties identified during interviews, but not studied extensively, are referred to as "named varieties". In both communes, the majority of farmers (88.9% in Tissa, 97.7% in Ourtzagh) cultivate *Beldi* (Table 2). The remaining barley is almost exclusively *Roumi.* There were no significant differences between communes for use frequency of *Beldi* (χ^2=2.37, df=1, p=0.12) or of *Roumi* (χ^2=1.04, df=1, p=0.31).

The majority of barley seeds of all varieties were maintained on-farm in both communes (Fig. 2). This proportion was lower in Tissa than in Ourtzagh (82% compared to 93%), although not significantly so (χ^2=2.18, df=1, p=0.14). The remaining seeds were mostly purchased or traded in markets. The distance from the villages to the nearest market varied from 0.1 Km to 14.6 Km. When considering only seeds of *Beldi*, the proportion of seeds maintained on-farm was nearly identical (93% in Tissa and 92% in Ourtzagh) in both communes.

The major reason cited by farmers in both communes for preferring to cultivate *Beldi* was "high productivity" (67% and 64% in Tissa and Ourtzagh, respectively) (Table 3). In Ourtzagh, the second most important reason was "resistance to drought"(31%); this was significantly higher than in Tissa (only 8.5%) (χ^2=5.13, df=1, p=0.02). Conversely, farmers in Tissa cited "cost

effectiveness" (50%), which was significantly more than farmers citing this reason in Ourtzagh (24%) (χ^2=4.66, df=1, p=0.03). Similar proportions of farmers in both communes cited local adaptation as a factor in their variety choice (approximately 21%).

The use made of the barley harvest in both communes was principally as animal fodder (both grain and vegetative biomass) and as seed for planting the following year (Table 4). A high proportion of farmers (58% and 63% in Ourtzagh and Tissa, respectively) sold a portion of their seed production in local markets. Barley was used for human consumption by 33% and 42% of farmers in Ourtzagh and Tissa, respectively. Some farmers reported setting aside a portion of the harvest specifically for use as seed in the following year. This practice was significantly more common in Ourtzagh (21%) than in Tissa (4%) (χ^2=4.02, df=1, p=0.04).

On-farm interviews, 2009

Farmers in all three communes cultivated barley in a number of small plots (less than one hectare in size), with total cultivation area and plot size being larger in Tissa than in Galaz or Ourtzagh (Table 5). Farmers cultivated a total of six barley varieties or variety complexes, with *Beldi* dominating cultivation in all communes (97%, 95% and 87% of cultivation area in Galaz, Ourtzagh and Tissa, respectively) (Table 6). Farmers did not report the use of variety mixtures as a strategy to increase yield or yield stability. The modern variety *Roumi* was the second most important cultivar, followed by *Rouiza* (a 2-row variety whose name can be translated as "small grain"), while the remaining three varieties (six-row

Rouiza, *Touinssi roumi* and *Stati*) were extremely rare, each being reported by only one farmer. The names attributed to these rare varieties provide some indication of their characteristics and/or origin. Six-row *Rouiza* refers to a small-grained variety with six rows. *Touinssi roumi* denotes a foreign variety of Tunisian origin and *Stati* refers to a variety originating from the town of Settat, in the Gharb valley region of Morocco. Although the current varietal richness of six is relatively high, values for Simpson's diversity index, which represents the probability that two randomly selected individuals will belong to the same variety, were also high, indicating low diversity. This is due to the dominance of *Beldi*. Tissa had the highest varietal diversity and richness of the three communes. A comparison of varieties currently cultivated and varieties no longer cultivated (Table 7), showed that most farmers in all three communes (over 95%) currently cultivate *Beldi*. The use of *Roumi* is increasing in all three communes, with more farmers currently cultivating *Roumi* than farmers who report discontinuing use of *Roumi*. Conversely, use of *Rouiza* is decreasing in all communes, with much higher percentages of previous use than of current use. There is also evidence of turnover in the varieties available in the region, with six-row *Rouiza*, *Touinssi roumi* and *Stati* representing newly introduced varieties while a free-threshing variety named *Chaïr Nabi* (i.e., the Prophets' barley") and a variety named *Zbiti* represent varieties that were previously cultivated in the region but are no longer available.

In the case of *Beldi,* the majority of farmers in all three communes report that they have been cultivating it "for a long time" (Fig. 3a), often stating that they inherited their *Beldi* seeds from their parents. Many farmers in each commune

(between 25% and 60%) report that they never renew their seed stock of *Beldi* from outside sources (Fig. 3b). This was the most frequent response in both Ourtzagh and Tissa communes, although in Galaz commune the majority of farmers report renewing their seed every two years. The remaining farmers reported periodically using outside seeds to renew or supplement their seed supply. These responses were mostly distributed between seed renewals every year to every five years, indicating relatively frequent influxes of new seed. Some farmers (2-12%) also indicated that frequency of seed renewal depends on outside factors and, as such, is variable.

Farmers report a number of reasons for renewing their seeds of *Beldi* from outside sources (Table 8). The most frequent reasons cited included that the farmers' seeds were mixed with seeds of other, undesirable, species (i.e., weeds), the seed supply was insufficient, or the seeds had poor germination. The number of farmers citing insufficient seed supply was significantly higher in Galaz than in Ourtzagh or Tissa (χ^2=7.104, df=2, p=0.03). On average, for each farmer, 70-90% of the *Beldi* seeds planted in 2009 came from stocks that had been maintained on-farm ("self") (Fig. 4). The remaining seeds were sourced from local markets, neighbours, seed companies, or non-local markets. There were no significant differences among the three communes in the percentage of seeds obtained from these different sources.

The seed exchange network in 2008 and 2009

Overall, the seed exchange network is similar in each of the three communes studied and is also similar in both years studied. The *Beldi* variety

complex has been cultivated for a long time in the region and is maintained almost exclusively through an informal seed exchange network, with very few seeds purchased from seed companies. Farmers in all communes produce most of the seeds used for planting on-farm (over 90% in 2008 and over 75% in 2009). Most of the remainder of seed is purchased in local markets. A relatively small proportion of farmers reserve a specific portion of the harvest for use as seed in the following year, but most do not reserve seed specifically for this purpose and simply plant the new crop using the seed remaining from the previous year.

The reasons cited by most farmers for obtaining seed from outside sources reflect problems with their own seed source, such as having insufficient seeds, a seed stock that is contaminated with too many weed seeds, or poor germination. There does not appear to be a conscious choice to renew their seed from outside sources in the hopes of obtaining new adaptive traits or to increase the diversity of their own seed stock. Although many farmers report never needing to use seed from outside sources, many others report using outside seeds, mostly at intervals varying from every year to every five years.

Genetic diversity, gene flow and genetic structure in *Beldi*

In samples of *Beldi,* between 6 and 8 alleles were identified for each SSR locus (Table 9). Values of gene diversity were high, and similar in both communes with most values being above 0.6. Similarly, allelic richness was nearly identical between Tissa and Ourtzagh, especially when rarefaction analysis was used to compensate for differences in sample size between communes (approximately 4.8 alleles per locus are expected in a sample size of N=30 for each commune). Private alleles were present and there was similar private allelic

richness in each commune. Overall, private allelic richness was relatively low, with approximately one private allele per locus expected in samples of the same size from each commune. Observed heterozygosity was extremely low, as is expected for a crop with an inbreeding mating system (results not shown). F_{ST}-values between villages were not significant (results not shown). In contrast, the F_{ST}-value measuring genetic differentiation between communes was low, but significant (F_{ST}=0.031, p=0.02). The number of migrants per generation between the two communes as calculated from the F_{ST} value was very high (N_em= 7.81). Similarly, results of AMOVA found a small (1.9%), but significant proportion of variation partitioned between communes, whereas the variation partitioned among villages within a commune (12%) was not significant (Table 10). The majority of variation (86%) was present within villages within communes, indicating high diversity maintained in each village. Bayesian analysis of population migration rates between communes using Ima2 identified similar and high levels of gene flow (with 95% confidence intervals) occurring in both directions between the communes ($M_{T\rightarrow O}$= 2.12 (0.28, 9.14) and $M_{O\rightarrow T}$ =2.82 (0.80, 8.60)). Analysis with MIGRATE gave values for population migration rates similar to those obtained using Ima2 ($M_{T\rightarrow O}$= 1.53 (0.05, 8.77) and $M_{O\rightarrow T}$ =2.13 (0, 9.29)). The rates of mutation-scaled migration ($m_{T\rightarrow O}$= 1.08) and $m_{O\rightarrow T}$ =1.72) in the MIGRATE analysis were similar to the mutation scaled population-sizes, (θ_{Tissa}= 1.78 and $\theta_{Ourtzagh}$= 2.54) indicating extremely high rates of historical gene flow between the two communes.

The most probable number of clusters identified using STRUCTURE was K=4. A relatively high proportion of genotypes from Tissa (frequency =0.421) were assigned to cluster 3, while genotypes from Ourtzagh were more likely to be assigned to cluster 1 (frequency=0.374) or cluster 4 (frequency=0.337) (Fig. 5). Fewer genotypes overall were assigned to cluster 2, but it was much more frequent in Tissa (frequency=0.151) than in Ourtzagh (frequency=0.052). These clusters indicate the presence of some geographic structure between communes, but there is also considerable overlap between communes, with some individuals in each commune assigned to each of the four clusters.

Combined results of interview and genotype data

The combination of interview data and genotypic data results in a more comprehensive depiction of seed exchange networks in Ourtzagh and Tissa (Fig. 6). Because the Ima2 model conforms more closely to our data than the MIGRATE model, we have elected to report the Ima2 values in the figure representing gene flow in the seed exchange network (Fig. 6). Although interview data identified the likely presence of gene flow among farms within a commune, genotypic data identified, in addition, a high level of gene flow between communes that was not reflected in the interview data.

Discussion

Diversity of barley in the study region

In all three communes, the diversity of the named barley varieties identified in interviews is relatively low because a complex of morphologically similar traditional varieties identified by the name *Beldi* occupies most of the cultivation area. For this reason, we have focused primarily on *Beldi* in our

analyses and discussion. A variety named *Beldi* in this region generally represents a traditional variety that has been cultivated by most farmers for so long that they are unable to report the specific time when they started cultivating it. Most farmers report obtaining their original stock of *Beldi* seeds from their parents, further confirming that varieties identified by this name have been present in the region for a long time. Farmers value *Beldi* because it has high productivity in the region and is resistant to drought. As a result, *Beldi is* considered reliable, cost effective and of good market value. Furthermore, the *Beldi* plant has a higher proportion of vegetative biomass than *Roumi* and therefore provides good quality animal fodder, which is a major use of the barley crop in the region, even more important than use as human food.

Beldi populations on individual farms have high genetic diversity and low structure, with each village containing a large proportion of the diversity of the population as a whole. This is similar to results for barley in other agro-ecosystems. In central Morocco, an evaluation of traditional varieties using storage proteins (hordeins) as markers also identified high diversity (Dakir et al., 2002). In Eritrea, a study using SSR markers also found high diversity and low structure, with the average individual field retaining 97% of the diversity of the population (Backes et al., 2009). By contrast, in Tunisia, barley varieties also have high diversity, but show some structuring according to geographic origin (Raoudha et al., 2007).

The large percentage of farmers cultivating *Beldi* indicates that farmers are maintaining a high use and valuation of *Beldi*, despite the availability of other named varieties, including varieties from the formal breeding sector. However,

the slight increase in the use of *Roumi* in all communes indicates that *Roumi* has replaced *Beldi* in a small portion of the barley cultivation area. Further studies of this region in future years will be necessary to determine if use of *Roumi* will continue to increase, and potentially displace *Beldi*. We believe it is likely, however, that *Beldi* will remain important in this region. The continued use of traditional varieties despite the availability of other varieties has been observed in other major crops, including maize in Mexico (Perales et al., 2003) and wheat in Turkey (Brush and Meng, 1998). In these cases, genetic erosion of traditional varieties is inhibited because the local varieties are uniquely adapted to both agro-ecological conditions and cultural needs (Bellon, 1996). As a result, their role in the local agro-ecosystem is not easily replaced and they provide better food security than most varieties from the formal breeding sector. This is particularly the case for barley in Morocco, where the different parts of the plant fill a large number of niches in traditional Moroccan farming systems. The grain provides both fodder for livestock (80% of production) and food for humans (20% of production) (Ceccarelli et al., 2001). In addition, the quality of barley straw is important because it provides livestock feed during the dry season (Rhrib and Amri, 2005) and can represent a significant proportion of the crop value (Annicchiarico and Pecetti, 2003). *Beldi* typically has better quality straw and much more straw biomass. Farmers in this region also practice "green stage grazing", allowing livestock to graze the barley plants in the early stages of growth and then allowing the plants to recover to produce tillers and grains (Belaid and Morris, 1990; Johnson, 1996). This is an advantageous practice that can increase the overall biological yield of the crop by 1000-2000 kg annually

without reducing grain yield (Yau, 2003). Very few modern cultivars can perform as well as traditional cultivars under this practice (Anderson, 1985; Yau and Mekni, 1987). These multiple uses are reflected in farmer selection criteria, which differ from formal breeding sector criteria that prioritize semi-dwarf, high grain-yielding varieties (Ceccarelli et al., 2001; Yau, 2003).

The seed exchange network for *Beldi*

By combining interview data with genotypic data, we have obtained a more comprehensive characterization of genetic diversity and gene flow within the seed exchange network than with either method used alone. Because of this, we have focused our discussion of the seed exchange network for *Beldi* in Tissa and Ourtzagh communes, where both types of data are available. In each commune, in a given year, approximately 10-20% of the seed supply is purchased in local markets, while the remainder of the seeds are from the farmers' own seed supplies. These influxes of seed should lead to a moderate level of gene flow within the commune.

There are some differences in the valuation and conservation of seed for *Beldi* between Tissa and Ourtzagh, that may be related to the differences in agroecological conditions between the two communes. In particular, the marginal conditions in Ourtzagh may increase vulnerability to drought, which may explain the much higher number of farmers in Ourtzagh who cite drought resistance as a reason for cultivating *Beldi.* Farmers in Ourzagh were also much more likely to reserve a portion of their seeds for planting the following year. This could be because they are more likely to experience low yields (due to smaller cultivated

areas, poor soil, and higher vulnerability to drought), and have therefore adopted this practice to help mitigate against years with a poor harvest.

The use of outside seeds does not appear to be higher in 2008 (the year following a poor harvest after a drought year in 2007) than in 2009 (the year following a good harvest). This is likely because some seeds from the 2006 harvest remained from the harvest in the year prior to the drought and these were used to plant the 2008 crop. In Ourtzagh, this may be partly due to the farmer practice of reserving a portion of the harvest specifically for planting. This conservation of seeds for planting was also undoubtedly helped by aid provided to farmers by the Moroccan government after the drought. Free sacks of barley were distributed to farmers through the local agricultural extension offices. Farmers used these seeds to feed their livestock, allowing them to retain their own seed for planting. If, however, the government does not provide assistance after a drought, or there are poor harvests for two or more subsequent years, seed reserves may be exhausted, leading to higher influxes of outside seed.

Based solely on the results of interview data, gene flow appears to be restricted to the scale of the commune because it occurs mainly between the local market and farms within the commune. There is also a small influx of seed from the formal breeding sector (seed companies) in the Tissa commune. However, the very small volume of seed from the formal sector is indicative of the overall dominance of seed exchange networks as the main source of seed.

Data from molecular markers indicate that gene flow is also occurring on a larger scale than the commune, with a high number of migrants between *Beldi* populations in the two communes, as indicated both by classical population

genetic tests and estimates of gene flow using Bayesian methods. High gene flow between communes is also corroborated by the low private allelic richness in each commune. This gene flow between communes is high enough that genetic structure between communes, although present, is weak.

Because the interview data do not directly indicate the cause of gene flow between communes, we must consider a number of different explanations. Gene flow between communes could occur through three potential routes: (1) vendors who sell seed in both of the local markets, (2) farmers from one commune travelling to the market in the other commune to purchase seed, or (3) farmers from different communes purchasing or trading seeds amongst themselves. It is also possible that the gene flow detected represents the signature of common ancestry of the *Beldi* populations in both communes, rather than ongoing gene flow, though this seems a less likely explanation because our model distinguishes population isolation from gene flow.

We believe that route (1), vendors selling seed in more than one local market, is most likely to account for gene flow. This type of gene flow would be facilitated by the structure of traditional rural markets in Morocco. These markets are periodic, i.e., they convene once a week in each market place, and the day of the week for the markets in a region are staggered, allowing one vendor or one farmer to attend more than one market. Historically, the markets have attracted vendors and buyers from a small radius of approximately 20-30 kilometers due to poor road conditions (Mikesell, 1958). However, with improvements in roads and transportation (Buerli et al., 2008), this radius may have expanded, resulting in increased long-distance gene flow, although recent data are not available. This is

likely the mechanism for high gene flow between communes, and suggests that genetic structure in the region may have decreased in recent years, compared to times when travel was more difficult and vendors would have been limited to fewer markets. The low genetic structure of *Beldi* among communes is therefore consistent with the levels of seed exchange and gene flow reported by farmers, given that market seed is likely to represent seed from a broader region than the commune. Similarly, a study of traditional maize varieties in Mexico (an outcrossing species), found that a reported 5% influx of seed per year from outside the community was sufficient to obscure any evidence of neutral genetic structuring between communities (Perales et al., 2005).

The other two explanations for gene flow among communes (farmers travelling to markets in other communes and farmers obtaining seed from farmers in other communes) are less likely to be significant because they would have been identified in the farmer interviews. On the questionnaire, farmers had the option to identify either "local markets" or "non-local markets" as the source of their seed, so farmers purchasing from non-local markets as a source of gene flow would have been detected during the interviews. Similarly, on the questionnaire, farmers had the option to identify "friend or relative from another village" as a source of seed and this was not reported, making it unlikely that significant levels of gene flow occurred via direct trade between farmers in different communes. Gene flow between communes occurring due to vendors selling in both local markets, however, was not accounted for in the survey questions.

The seed exchange network for barley appears to be similar to that for fava bean (*Vicia faba* L.), a legume crop that is cultivated in rotation with barley in the

region. In the case of *V. faba,* most seed exchanges happen locally, between neighbours or relatives in the same area. However, some seed exchanges happen on a larger scale, when farmers purchase from vendors representing a larger geographic area (Arbaoui, 2003). It is therefore likely that these two interconnected crops share similar patterns of genetic diversity and genetic structure.

A recent study of traditional barley varieties in high-hill agro-ecosystems in Nepal identified levels of seed exchange for two different barley varieties similar to those reported in this study, with 20-25% of barley seeds obtained through exchanges with other farmers in a given year (Bajracharya et al., 2012). This, coupled with frequent renewal of seed stocks following crop failure led to a similar lack of genetic differentiation, particularly in marginal and poor environments. The parallels between our findings and those for barley in Nepal likely reflect the similar environments in which traditional barley varieties are cultivated, i.e., agriculturally marginal environments that are unsuitable for higher value crops such as wheat or rice.

Conservation implications

Close to 100% of barley seed stocks in the region are managed via the seed exchange network with very few inputs from the formal seed sector. The seed exchange network is therefore critical to barley supply and diversity in the region, even more so than for other cereals in Morocco (mainly wheat) where it accounts for 89% of seed (Van Mele et al., 2011). As a result, any vulnerability in the seed exchange network will likely have an impact on the diversity and evolution of *Beldi*.

This study highlights some vulnerable points in the seed exchange network that should be considered in the context of crop yield stability and food security in the region, as well as for *in situ* conservation of barley diversity in this region. In particular, the small proportion of farmers who set aside seed specifically for use in planting the following year is a cause for concern because in the case of a year (or a series of years) with a poor harvest (e.g., due to drought, fungal disease, or insect pests), farmers may use all their seeds prior to planting time, and may then be forced to purchase seeds from outside sources. These seeds may not be of high quality or may come from a different region and be poorly adapted to local conditions. This effect is currently mitigated by government aid programs that provide additional seeds to farmers for feeding their livestock after a poor harvest, allowing farmers to retain some of their own seeds for planting in the following year.

Even in a year with a good harvest, however, the remaining seeds from the previous harvest that are used for planting the new crop may not be the highest quality seeds, resulting in poor germination, or poor yield. This vulnerability in the seed system is already apparent in the number of farmers who periodically renew their seed supply from outside sources because their own seed is insufficient or of poor quality rather than because of a deliberate choice to acquire new seeds. By comparison, in other seed exchange networks, there are frequent reports of farmers deliberately choosing to renew their seeds in search of new traits (e.g., culinary properties, early maturity), to increase quality and yield, or as a component of a social ritual (e.g., a gift or exchange at a wedding) (e.g., Asfaw et al., 2007; Delaunay et al., 2008).

Additional factors that cause decreased yields and poor quality harvest, such as climate change, are likely to compound this vulnerability in the seed system and may have an influence on the continued evolution of *Beldi* in the region (Pautasso et al., 2012). Morocco has been subject to drier conditions and prolonged periods of drought since the 1980's (Esper et al., 2007). In this context, it is especially important for farmers to reserve a portion of their stored seed for planting the crop in the following year. Additional interventions that can help mitigate loss of seed stores due to adverse environmental conditions include community seed banks, diversity-kits, diversity fairs, and micro-credit schemes (Jarvis et al., 2011). Any strategies that involve the development of new community seed storage facilities will, however, require good design and monitoring to avoid seed loss to pests (e.g., insects and rodents) and/or poor storage conditions (e.g., excess heat and humidity).

Future research directions

This study points to a number of questions that merit further study. The combined use of interview data and molecular markers has identified local markets as a potential source of cryptic long-distance gene flow. In future studies, assessing the diversity and sources of seeds available in local markets would provide valuable information regarding sources of gene flow. Also, although we only detected weak genetic structure on the basis of neutral molecular markers, it is possible that markers for adaptive traits or loci would show some structuring across an agro-climatic gradient. Further research should therefore investigate differences in adaptive traits, in addition to neutral markers. It is also possible that we did not detect strong structure because the spatial scale investigated was too

small and future studies might detect stronger barriers to gene flow if samples are collected from a much larger geographical area.

Here, we have identified *Beldi* as a traditional variety complex that has been cultivated in the region for a long time. A suite of common morphological characteristics is consistently associated with the varieties identified as *Beldi* in the study region. However, because the variety name simply means "local", it is possible that the variety considered to be local has changed over time, but the name has remained the same. Work on traditional crop varieties in Morocco has demonstrated that, with increasing geographic distance, varieties with the same names tend to differ morphologically (Sadiki et al., 2007). It is likewise possible that with increasing time, varieties with the same name will also differ morphologically. Further work using samples collected at previous times and stored in seed banks will be required to ensure that the same morphological variety is being referred to and that *Beldi* is, in fact, representative of varieties that have historically been cultivated in the region.

Conclusions

Future studies of barley in this region should focus more extensively on the complex of varieties identified as *Beldi,* given the high importance and valuation of *Beldi*, rather than on the other named varieties identified in this study. This will allow better understanding of the nature of this variety complex, in particular the extent of morphological divergence among different populations of *Beldi*. The use of a combination of interview and molecular data in the study of traditional crop varieties allows a more comprehensive assessment of gene flow via the seed exchange network than either method used alone. In this case,

combining both methods allowed us to identify moderate to high levels of gene flow both within and between communes. It is advisable to combine both types of data, particularly in systems that are not yet well characterized. Insights obtained using this approach may then be used to refine future studies and conservation strategies. Finally, the seed exchange network in this region is likely to be vulnerable to years with poor harvests, which may be compounded by the effects of climate change and periodic drought. Efforts at *in situ* conservation of barley in this region should aim to increase the proportion of farmers and/or communities maintaining a reserve supply of seeds.

Tables

Table 1. Villages in Ourtzagh and Tissa communes where farmer interviews were conducted and barley seed samples were collected in 2008.

Rural Commune	Village (*douar*)	Elevation (m)[a]	Distance to market (Km)[a]	$N_{int.}$[b]	N_{ssr}[c]
Ourtzagh	Ain Bouchrik	515.6	3.3	4	--
Ourtzagh	Ain Chajra	169.1	2.2	4	8
Ourtzagh	Ain Kchir	200.6	11.0	4	--
Ourtzagh	Beni Moumen	178.9	6.1	4	12
Ourtzagh	Benihelal	505.8	5.4	4	15
Ourtzagh	Boubiad	--	11.7	6	--
Ourtzagh	Bouferrah	235.2	8.4	4	--
Ourtzagh	Koudia	--	--	5	--
Ourtzagh	Sidi Snoun	273.6	11.0	4	--
Ourtzagh	Tcharou	212.6	10.4	4	12
Tissa	Abdejlalat	151.3	14.6	2	--
Tissa	Bratla (Ras El Oued)	190.9	0.1	2	--
Tissa	Coop. Alaouia	212.8	6.4	4	12

Rural Commune	Village (*douar*)	Elevation (m)[a]	Distance to market (Km)[a]	$N_{int.}$[b]	N_{ssr}[c]
Tissa	Coop. Youssoufia	155.4	8.6	3	--
Tissa	Gaada	--	--	2	8
Tissa	Hammoudiate	238.1	7.0	3	--
Tissa	Hashimia	216.4	6.7	4	--
Tissa	Jiahna	174.6	8.8	3	10
Tissa	Massakine	184.2	4.1	4	7

[a] Calculated from GPS coordinates. Missing values are due to a GPS malfunction.

[b] The number of farmers interviewed.

[c]The number of samples genotyped using SSR markers. Four villages per commune were randomly selected for sample genotyping among the villages visited during interviews. Three or four seeds were genotyped from each sample selected.

Table 2. The percentage of farmers cultivating the different barley varieties (or variety complexes) in Ourtzagh and Tissa in 2008.

	Farmers using each variety (%)		
Commune[a]	***Beldi* (TV)**[b]	***Roumi* (MV)**[c]	***Rouiza* (MV)**[c]
Ourtzagh	97.7	2.3	0
Tissa	88.9	7.4	3.7

[a]There were no significant differences between communes for *Beldi* in a Chi-squared test (χ^2=2.37, df=1, p=0.12) or for *Roumi* (χ^2=1.04, df=1, p=0.31).

[b] TV refers to a traditional variety or variety complex.

[c] MV refers to a modern variety or variety complex.

Table 3. Percentage of farmers citing specific reasons for prefering to use *Beldi* over other varieties in 2008.

	Commune	
Reason for selecting *Beldi*	**Ourtzagh**	**Tissa**
Productivity	64.3	66.7
Resistance to drought	31.0*	8.3*
Cost effectiveness	23.8*	50.0*
Local adaptation	21.4	20.8
Fodder quality	19.0	8.3
Personal habit	11.9	8.3
Resistance to biotic stress	9.5	4.2
Cooking quality	7.1	12.5
Cost	7.1	4.2
Market value	0.0	12.5

*Indicates a significant difference in a Chi-squared test ($p<0.05$)

Table 4. Percentage of farmers citing specific postharvest uses for the barley crop in 2008.

	Commune	
Harvest use	**Ourtzagh**	**Tissa**
Fodder	95.2	87.5
Grain	95.2	83.3
Sale	59.5	62.5
Food	33.3	41.7
Seed reserve	21.4*	4.2*

*Indicates a significant difference in a Chi-squared test ($p<0.05$).

Table 5. Number of barley plots, plot size and barley cultivation area per farmer in 2009.

Commune	**Total area surveyed (Ha)**	**Mean no. barley plots/farmer (SD)**	**Mean plot size in Ha (SD)**	**Mean cultivation area (Ha) per farmer (SD)**
Galaz	80.80	2.43 (1.17)	0.55 (0.47)	1.40 (1.20)
Ourtzagh	47.80	2.26 (1.09)	0.49 (0.46)	1.11 (0.87)
Tissa	110.18	2.90 (1.54)	0.77 (0.65)	2.01 (1.47)

Table 6. Percentage of the total barley cultivation area in each commune devoted to the different barley varieties or variety complexes cultivated in 2009.

	Cultivation area devoted to each variety (%)						
Commune	***Beldi*** **(TV)[a]**	***Roumi*** **(MV)[b]**	***Rouiza*** **(MV)**	**6-row *Rouiza*** **(MV)**	***Touinssi roumi*** **(MV)**	***Stati*** **(MV)**	**Simpson's diversity index**
Galaz	96.9	2.7	0.5	0	0	0	0.94
Ourtzagh	94.8	4.4	0.8	0	0	0	0.90
Tissa	86.7	7.3	0.8	2.5	1.8	0.9	0.76

[a] TV refers to a traditional variety or variety complex.

[b] MV refers to a modern variety or variety complex.

Table 7. Percentage of farmers presently cultivating and previously cultivating (i.e., no longer cultivating) the reported barley varieties or variety complexes in 2009.

	Galaz	**Galaz**	**Ourtzagh**	**Ourtzagh**	**Tissa**	**Tissa**
Variety name	**Current**	**Previous**	**Current**	**Previous**	**Current**	**Previous**
Beldi	96.7	0	95.5	2.3	95.9	4.1
Roumi	11.7	8.3	15.9	6.8	14.3	4.1
Rouiza	3.3	21.7	4.6	9.1	4.1	12.2
Chaïr nabi 6-row	0	3.3	0	3.3	0	0
Rouiza	0	0	0	0	1.7	0
Touinssi roumi	0	0	0	0	1.7	0
Stati	0	0	0	0	1.7	0
Zbiti	0	1.7	0	0	0	0

Table 8. Percentage of farmers citing specific reasons for renewing their seed supply of *Beldi* from outside sources in 2009.

	Commune		
Reason for renewal	**Galaz**	**Ourtzagh**	**Tissa**
Insufficient seed supply	43.1*	23.8*	21.3*
Presence of weed seeds	29.3	21.4	12.8
Poor seed germination	8.6	14.3	2.1
To increase yield	12.1	0	4.3
Seeds had lost resistance	12.1	0	0
Poor seed quality	6.9	0	0
To reduce disease	5.2	0	0
To renew the seeds	3.5	4.8	4.3
Seeds were diseased	3.5	2.4	0
No reason given	3.5	2.4	0

*Significantly different in a Chi-squared test ($p<0.05$)

Table 9. Gene diversity, number of alleles, allelic richness, and private allelic richness of *Beldi* in Tissa and Ourtzagh (Ourt.) communes. Numbers in parentheses represent the number of private alleles for each locus.

	No. of alleles		**Gene diversity**		**Allelic richness[a]**		**Private allelic richness[a]**	
Locus	**Tissa**	**Ourt.**	**Tissa**	**Ourt.**	**Tissa**	**Ourt.**	**Tissa**	**Ourt.**
Bmac0316	4(1)	5(2)	0.6	0.7	4.0	4.6	1.3	1.9
Bmag0013	6(1)	7(2)	0.8	0.8	5.9	6.1	1.5	1.8
Bmag0125	5(1)	6(2)	0.7	0.5	4.9	4.8	1.5	1.4
Bmag0321	5(2)	4(1)	0.6	0.6	4.4	3.3	1.5	0.5
Ebmac0541	5	5	0.8	0.7	5.0	4.7	0.3	0.0
Bmag0345	5	6(1)	0.7	0.7	4.4	5.3	0.1	1.0
Total[b]	-	-	0.7	0.7	4.8	4.8	1.1	1.1

[a]Adjusted for sample size using rarefaction.

[b]Average over loci

Table 10. Partitioning of variance between different hierarchical levels of sampling as determined by AMOVA.

	Percent of variation		
Locus	**Between communes**	**Among villages within communes**	**Within villages within communes**
Bmac0316	4.82390*	21.7602**	73.41589*
Bmag0013	-2.23766	21.74040**	80.49726
Bmag0125	5.22475	12.38034	82.39492
Bmag0321	0.75996	4.20511	95.03493
Ebmac0541	2.50659	4.83007	92.66334
Bmag0345	0.92468	4.40354	94.67178
All loci	1.88163*	11.94537	86.17301***

*p<0.05, **p<0.01, ***p<0.001 (1023 permutations)

Figures

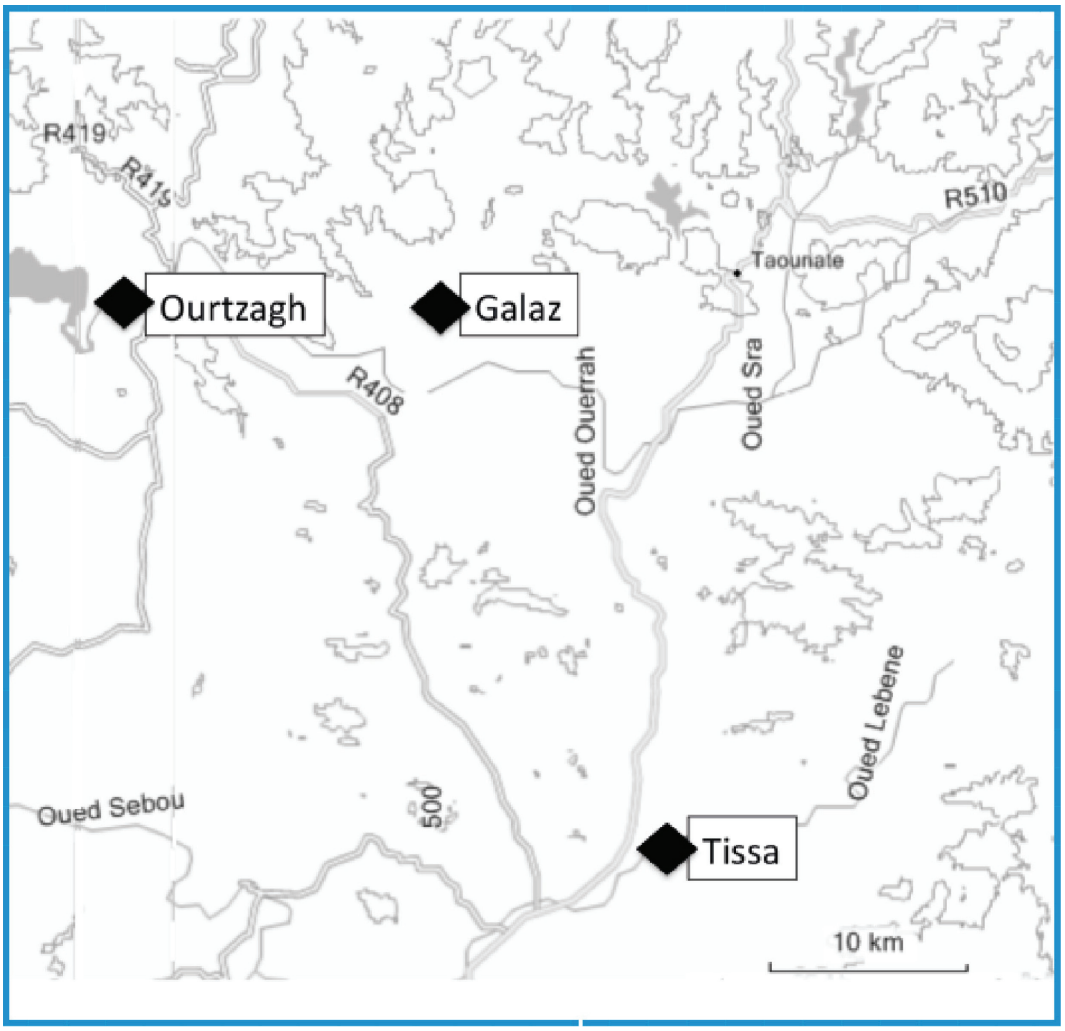

Figure 1. The administrative centers of the three rural communes of Taounate Province (Tissa, Ourtzagh and Galaz), where interviews and/or seed sampling were conducted in 2008 and 2009.

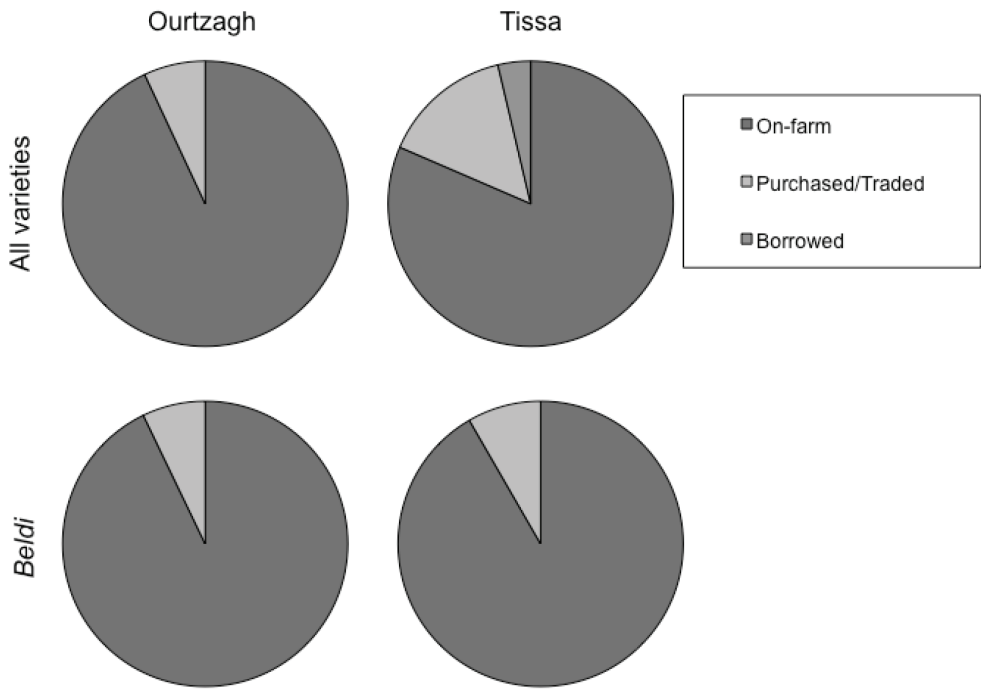

Figure 2. The sources of the seeds used for planting in 2008 in the rural communes of Ourtzagh and Tissa. Data are presented for all varieties combined (top) and for the *Beldi* variety complex only (bottom). The majority of seeds were maintained on-farm, but a small proportion of seeds was obtained from outside sources in both communes. The seeds obtained from outside sources in Tissa commune is higher for all varieties combined than for *Beldi* considered alone.

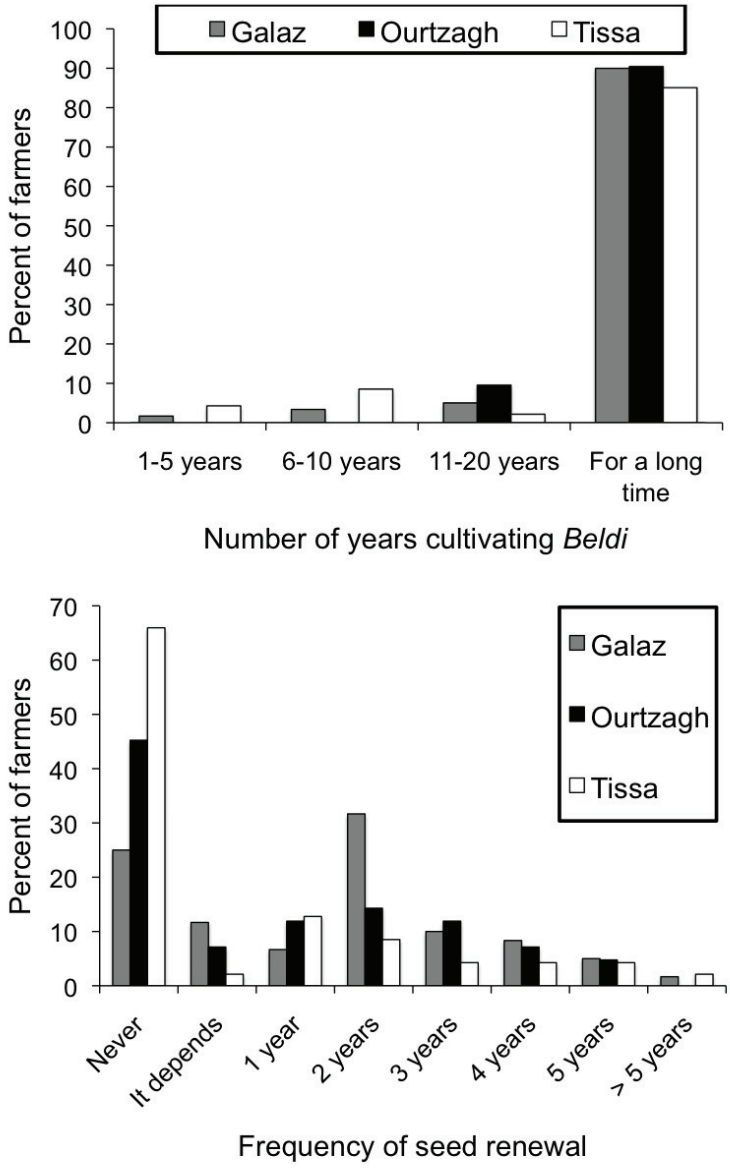

Figure 3. The patterns of use of *Beldi* over time represented as (a) the number of years that farmers report cultivating *Beldi* and (b) the frequency with which farmers renew their supply of *Beldi* seeds from outside sources. (Data from the 2009 interviews).

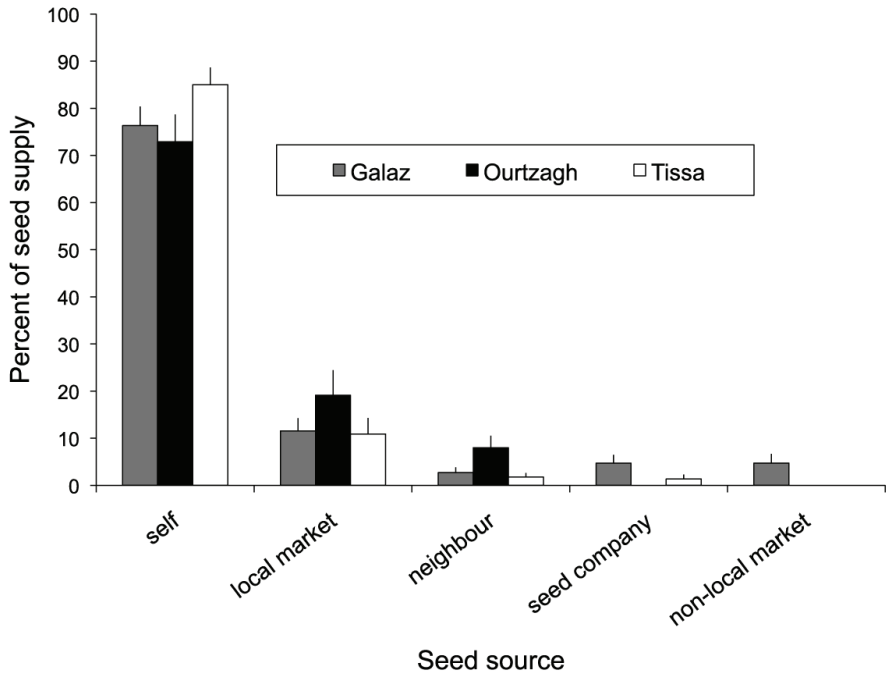

Figure 4. The sources of *Beldi* seeds used for planting in 2009 in the rural communes of Galaz, Ourtzagh and Tissa. The percentage of seeds obtained from each source is expressed as a percentage (+ SE). There are no significant differences among communes.

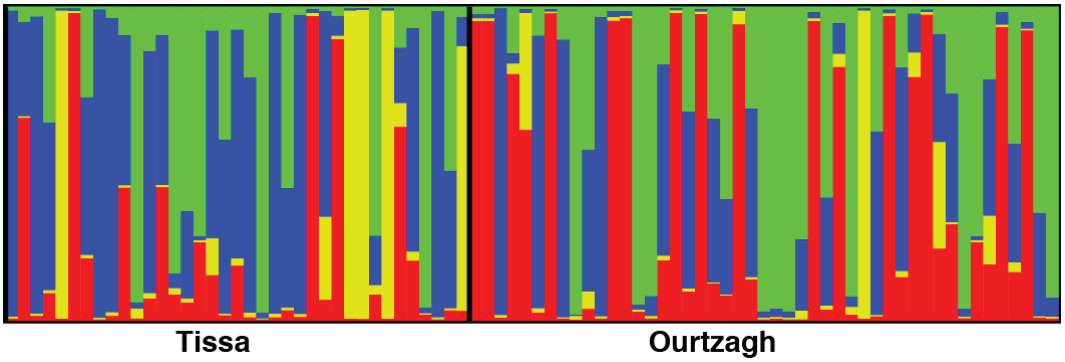

Figure 5. Population structure of *Beldi* seeds from Tissa and Taounate communes estimated using genotypic clustering in STRUCTURE for K=4 clusters (red=cluster 1, yellow=cluster 2, blue=cluster 3, green=cluster 4).

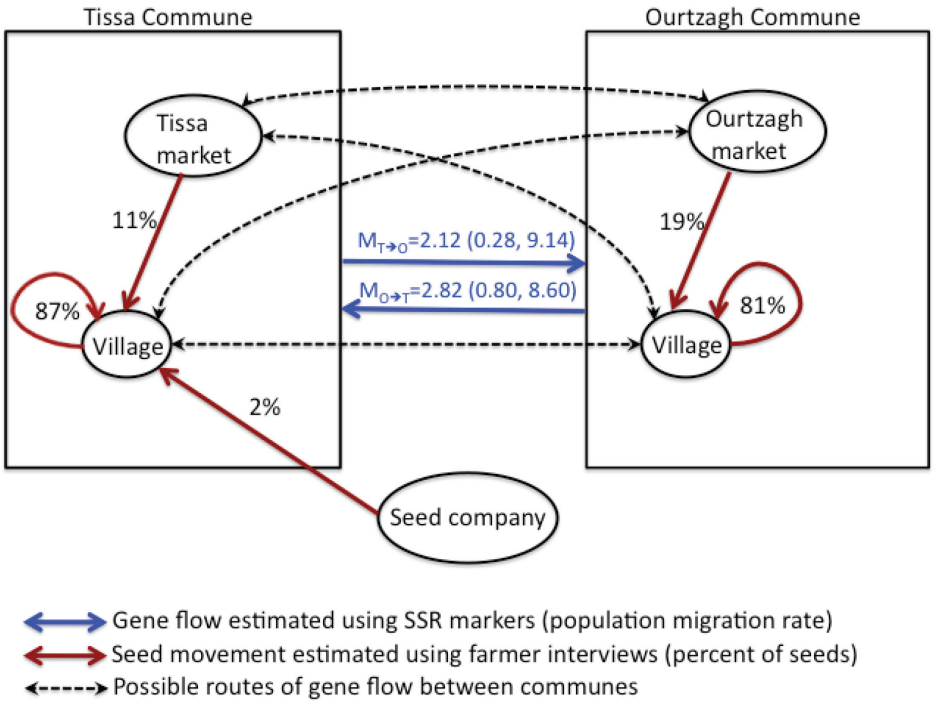

Figure 6. The *Beldi* seed exchange network and gene flow within and between the rural communes of Tissa and Ourtzagh. The sources of seed (indicated as percentages of total seed used in a year) are derived from interview data. The seeds sourced within the village include seeds renewed on-farm and seeds obtained from a neighbour in the same village. Population migration rates (the rate at which genes from one commune are supplanted by genes from the other commune) are calculated from SSR data using Ima2 and are reported as M values ($M=2N_em$) with 95% confidence intervals. Farmers in both rural communes report using over 80% of their own seed and buying the remainder from local markets within the commune.

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Linking statement

In Chapter 2, I reported that farmers in Taounate province of Northern Morocco predominantly cultivate a variety referred to as *Beldi* and that they maintain most of the seeds of this variety (70-90%) on farm and obtain new seeds from local markets, often because their own seed supply is damaged or insufficient, in particular after a drought year. This periodic replenishment of seeds suggests that, over time, the gene pool of the traditional variety may change, due to local extinction, migration and drift. I also suggest, in Chapter 2, that gene flow from markets represents a potential source for an influx of genes from a larger surrounding region because some vendors may sell in more than one market, thereby disseminating seeds to a large region. Therefore, it is possible that, over time, the farmer seed exchange network results in dynamic change of the gene pool of the barley crop and, potentially, a break-down of local genetic structure due to gene flow from markets. In Chapter 3 I use historical seed samples collected from farms in 1985 and compare them to contemporary seed samples collected at the same sites and also to samples of seeds purchased in local markets to identify changes in the genetic diversity, and genetic structure of barley populations, and to assess the extent of gene flow from markets to farm populations.

CHAPTER 3: Loss of genetic structure in a traditional barley variety in Morocco: the effect of market seed?

Jensen, HR, Sadiki, M, Schoen, DJ Loss of genetic structure in a traditional barley variety in Morocco: the effect of market seed? For submission to: Conservation Biology.

Abstract

An important part of the world's agricultural biodiversity is maintained in the developing world where farmers grow traditional crop varieties in many different environments. This type of conservation is dynamic, and factors such as regional development and climate change are likely to influence the conservation of this diversity. We studied spatial and temporal variation in the population genetic structure of a traditional variety of barley named *Beldi* in Northern Morocco, a region where both transport infrastructure and drought frequency and severity have increased significantly in the past decades. Microsatellite markers were used to genotype seed samples collected in 1985, seeds collected from the same populations in 2008, and seeds collected from local markets in 2009. Genetic structure was generally low, and we observed a significant decrease in genetic structure over time, with differences among populations accounting for 5% of the variation in 1985 and none of the variation in 2008. Seeds from 2008 had more similarities to market seed than seeds from 1985. Thus, despite the continued use of *Beldi* in the study region, there has been a loss of genetic structure, possibly attributable to increased gene flow from markets. We discuss the potential impact of these results on the ongoing evolution and local adaptation of *Beldi*.

Introduction

Traditional varieties of both major and minor crops are cultivated on large areas in many regions worldwide (Jarvis et al., 2008; Teshome et al., 1999; Barcaccia et al., 2002; Zhou et al., 2003; Barry et al., 2007; Pusadee et al., 2009). Crop conservation on-farm (*in situ*) is a dynamic process, with some varieties falling into disuse, and new varieties being adopted (Brush, 1989). Crop varieties maintained and selected on-farm are subject to the evolutionary forces of selection, genetic drift, and migration (gene flow) (Alvarez et al., 2005). Varieties of one crop can act as metapopulations, with local populations (farmer fields) subject to extinction and recolonization (van Heerwaarden et al., 2009).

The management of seed through informal seed exchange networks results in varying levels of gene flow within and among farmer households for a given variety. Migration within inbreeding crop varieties is almost entirely due to seed exchange between farmers and seed purchases in local and regional markets. The connectivity of crop variety populations as mediated by farmers and the opportunities for exchange of germplasm are therefore important structuring factors for the genetic diversity of cultivated varieties (Berthouly et al., 2009).

The use of seeds from local markets results in the periodic influx of migrant genes into local populations of traditional crop varieties. Despite this source of gene flow, traditional seed management by farmers has frequently resulted in genetic differentiation of traditional crop variety populations (Barry et al., 2007). This is probably due to limits on gene flow between isolated subpopulations (Pusadee et al., 2009) combined with relatively strong selection (both natural and artificial) for adaptation to the local environment (Horneburg

and Becker, 2008). Geographical isolation can limit the amount of seed exchange (and thereby gene flow) between regions, thus maintaining genetically distinct populations of traditional crop varieties (Alvarez et al., 2005). These populations sometimes follow a pattern of isolation by distance, as in the case for rice in Thailand (Pusadee et al., 2009), but factor such as proximity to urban areas and large markets, social networks, and climate can also affect the distribution of crop variety populations (e.g., Parzies et al., 2004).

Changes in migration patterns may occur rapidly as a result of changes in the seed exchange network that mediates distribution of seed, and have a strong influence on the spatial distribution of genetic diversity (van Heerwaarden et al., 2009). In many developing countries, regional development may alter previously established patterns of gene flow between regions that have helped to structure crop intravarietal diversity in the past (Lipper et al., 2012). Recently, the access of farmers in many remote areas to seeds from regions outside their own has improved through the development of transport infrastructure. As a result, there is potentially increased gene flow between different crop populations.

It is unlikely that the genetic diversity and geographic structure of traditional crop variety populations will remain constant in a changing environment. Although numerous local *in situ* conservation projects have been implemented, *in situ* conservation is not globally managed and monitored (Secretariat of the Convention on Biological Diversity, 2010) and we are unaware of the long-term effectiveness of on-farm conservation, particularly in a changing environment. An almost complete loss of traditional crop varieties has already occurred in Europe after the introduction of new agricultural techniques and crop

varieties during the 20th century, despite plans for *in situ* conservation dating back to 1927 (Zeven, 1996). If the changes in genetic diversity that result from changes in the movement of seeds between regions can be detected and the factors causing these changes can be identified, then programs to conserve agrobiodiversity on an appropriate eco/geographic scale will be easier to implement by allowing researchers to design sampling strategies for conserving representative local diversity.

Here we test the hypothesis that genetic structure and diversity have changed over time in a developing region, and we explore potential explanations for these changes. We studied the population structure of an inbreeding crop, barley (*Hordeum vulgare* L. ssp. *vulgare*), maintained on-farm over a broad geographic region in Northern Morocco, using data from two points, 1985 and 2008. Barley is an ancient crop that was domesticated over 10 000 years ago and has been cultivated in this region for several thousands of years (Meyer et al., 2012). Although most of the barley seeds that farmers plant are maintained on-farm, they sometimes purchase seeds from local markets when their own supply is insufficient or damaged (Jensen et al., 2012a). In particular we sought to answer the following questions: (1) What is the genetic diversity and structure of a traditional variety of barley in the study region in 1985 and in 2008? (2) Have genetic diversity and structure in the region decreased between 1985 and 2008? (3) What is the diversity and genetic structure of seed available in regional markets? (4) Is there evidence of gene flow from markets to farm populations?

Materials and Methods

Local variety identification and nomenclature

The major traditional variety name in the study region is *Beldi,* which translates as "local" (Jensen et al., 2012a). Because *Beldi* is a generic identifier, it could potentially refer to a complex of morphologically distinct local varieties, rather than a single local variety. For the purposes of this paper, however, we will refer to the samples of *Beldi* that we are working with as different populations of one variety, rather than as a variety complex, because all of the samples of *Beldi* that we collected could be classified as one variety based on morphology. The variety we refer to as *Beldi* is tall, with six-row spikes, lax spike density, awned grains, barbs on the lateral nerves of the lemma, lemma awn barbs present along the entire length of the awn, and white aleurone colour (Jensen et al., 2012b). This is consistent with suggestions that identification of traditional varieties should be based on both local nomenclature and a suite of morphological traits (Sadiki et al., 2007).

Sample collection: farmer seeds, 1985 and 2008

We selected 7 sites in the Northern Moroccan provinces of Tetouan, Chefchaouenne and Taounate to collect samples of *Beldi* (Table 1). These sites cover a geographically heterogeneous region spanning the Mediterranean coast, the Rif Mountains, and the Pre-Rif regions to the south. The selected sites have elevations ranging from 5 m to 1100 m, moisture regimes ranging from semi-arid to subhumid and winter types ranging from cool to mild (the winter type is particularly relevant because barley is cultivated from December to May in the study region). Assessments of moisture regime and winter type are based on the

agroclimatic zones defined by the United Nations Food and Agriculture Organisation) (FAO, 2006). We obtained seeds of traditional barley varieties previously collected from farmers in these sites in 1985 by researchers from ICARDA (International Center for Agricultural Research in the Dry Areas, Aleppo, Syria) and maintained *ex situ* in the ICARDA seed bank (Perrino et al., 1986).

In 2008 we re-sampled barley seed and/or leaf tissue from *Beldi* currently cultivated in these same sites. Barley seed and/or leaf samples in 2008 were collected within a 10 km radius of each original sampling location from 1985. Leaf tissue samples were preserved in silica gel. At the time of sample collection, a short interview was conducted with farmers to identify the name of the variety.

Sample collection: market seeds

Local markets are likely to be the main source of new germplasm and gene flow in the study region, with farmers reporting that they obtain most new seeds from markets, rather than from friends or relatives from other villages or from the formal seed sector (Jensen et al., 2012a). We sampled the *Beldi* seeds available in rural markets throughout the study region in 2009 to characterize the genetic diversity and structure of barley germplasm available in markets and to identify the possible sources of gene flow between populations of *Beldi*. We sampled the markets during the month of October because the barley crop is planted in November/December (depending on the date of the arrival of the rainy season) and it is therefore during this period that farmers will procure seeds from markets for the purpose of planting. We visited 12 markets distributed throughout the study region located at close proximity to the on-farm sampling sites (Table 2).

We purchased 1-2 Kg of *Beldi* seed from four to six different vendors in each market. Four or five seeds per vendor were used for genotyping in each market.

DNA extraction and SSR genotyping

For DNA extraction from seed samples, seeds were germinated and leaf tissue was collected from the primary leaf of the seedlings. Total genomic DNA was extracted from leaf samples using the DNA plant mini kit (QIAGEN, Toronto, Canada). Polymerase chain reaction (PCR) was used to amplify six unlinked microsatellite markers for these samples (Bmag0013, Bmag0321, Bmag0345, Bmac0316, Bmag0125, EBmac0541) (Ramsay et al., 2000). For each PCR reaction one primer was designed to contain an M13 tail and a second reaction incorporated a primer with an M13 sequence and end-labelled with an infrared dye with fluorescence at either 700 or 800 nm (Li-Cor Biosciences, Lincoln, NE, USA). Details of PCR conditions are described in Appendix III (Tables S2, S3). Genotypes were visualized and scored by running PCR products on acrylamide gels using a 4300 DNA Analyser (Li-Cor Biosciences, Lincoln, NE, USA). Bands at microsatellite loci were identified and sized using SAGA software (Li-Cor Biosciences, Lincoln, NE, USA).

Data analysis

For each group of samples (on-farm (1985), on-farm (2008) and market seed), we calculated genetic diversity parameters including the number of alleles per locus, gene diversity, allelic richness and private allelic richness using Arlequin 3.11 (Excoffier et al., 2005). We adjusted the values of allelic richness and private allelic richness using rarefaction for 10 genes using the program HP-

RARE V.1.1 (Kalinowski, 2005), to account for differences in sample sizes (Kalinowski, 2004).

To test for hierarchical partitioning of neutral genetic diversity within and among sites we conducted an analysis of molecular variance (AMOVA) using Arlequin 3.11 (Excoffier et al., 2005). The analysis was run separately on the samples from 1985, 2008 and markets in order to characterise the structure of genetic diversity at each time point. We then assessed differentiation between pairs of sites within each year using pairwise Fst-values.

To assess the differentiation occurring overall between 1985 and 2008 in the field sites, between 1985 field sites and market seed, and between 2008 field sites and market seed, we conducted 3 separate AMOVA analyses (using Arlequin 3.11) comparing variation between years, among populations within years, among individuals within populations and within individuals. To determine if population structure followed a pattern of isolation by distance, we conducted Mantel tests with samples from 1985, 2008 and markets using Arlequin 3.11.

We tested for the presence of clusters within samples using the Bayesian algorithms implemented in the program STRUCTURE (Pritchard et al., 2000). We ran the admixture model for an inbreeding species with a burnin of 100 000 and a run time of 100 000, five iterations, and cluster numbers from K=1 to K=6. We used the method of Evanno et al. (2005) and the STRUCTURE HARVESTER software (Earl and vonHoldt, 2012) to evaluate the most likely number of clusters in the data.

Drought severity data and barley yield

Environmental conditions have an impact on crop growth and yield, and therefore on the number of seeds produced and available to farmers. To assess the environmental conditions prevalent during the study period, and their potential impact on barley production, we used the self-calibrating Palmer Drought Severity Index (scPDSI) as an indicator of agricultural drought occurrence in the study region over a period of 52 years, from 1950 until 2002 (the last year for which data are available). The PDSI provides an indicator of drought severity using monthly data for precipitation, temperature, and locally available water content in the soil (Palmer, 1965). We used scPDSI values with a resolution of 0.5˚ X 0.5˚ from three grid points in Northern Morocco (5.25W, 35.25N (Chefchaouen); 5.75W, 35.25N (Larache); 5.75W, 35.75N (Tangier)) for the months of March and April ($scPDSI_{3\text{-}4}$) (Dai et al., 2004; van der Schrier et al., 2006). These are the months when grain filling occurs and moisture availability in these months is the principal determinant of crop yield, making this the most biologically relevant metric (Mavromatis, 2007).

Results

Genetic diversity, allelic richness and private allele richness remained similar in 1985 and 2008 (Table 3). Gene diversity remained similar in market seed compared to farm seed from 1985 and 2008, but allelic richness and private allelic richness were slightly lower in market seed compared to farm seed. The partitioning of neutral genetic variance within and among populations as measured using AMOVA changed between 1985 and 2008. In 1985, a significant

proportion of the variation (4.93%) was accounted for by differences among populations compared to 2008. In the latter year differences among populations accounted for none of the variation detected (Table 4). In both years, the majority of genetic variation was present among individuals within populations (92.2% in 1985 and 99.23% in 2008). The market seed showed a partitioning of variation that was similar to that seen in the 2008 farm sites, with variation among individuals within markets accounting for 96.19% of variation, and a non-significant value of variation among markets.

The results of site-to-site comparisons using pairwise Fst-values identified more site pairs with significant differentiation in 1985 than in 2008 (Table 5), consistent with the results of AMOVA, again indicating that differentiation between sites generally decreased with time. In the AMOVA analyses, farmer seeds from 1985 and 2008 were not significantly differentiated from one another, but both had significant differentiation from the market seed (11.50% and 11.95% of variation accounted for by this component in comparisons with 1985 and 2008, respectively) (Table 6). Although it may appear contradictory that seeds from 1985 and 2008 have differences in structure when considered individually, but are not significantly different from one another when compared directly, this is likely because when seed from each year is tested against a null of no structure there is greater statistical power to detect structure compared to testing the distributions of the two years against one another.

Farmer seeds from 1985 and 2008 did not show evidence of isolation by distance (Table 7), suggesting that at both of these time points there was a complex pattern of gene flow among populations that was not solely influenced

by the distance between sites. However, seeds from markets did have a significant pattern of isolation by distance (r^2=0.32, p=0.03) (Table 7), indicating that increasing geographic distance between markets does result in a different genetic composition of the seeds available for purchase.

When data are analyzed using STRUCTURE, the value of delta K indicates support for the presence of two clusters. We present the results of the highest probability run for K=2. Results are summarized as proportion of cluster assignment for each population in each year and presented on a map of the region (Fig. 1). There is a clear clustering of seeds from markets, compared to farm seed from both 1985 and 2008. Market seeds are more frequently assigned to cluster 1, whereas farm seeds are more frequently assigned to cluster 2. There is, however, a higher proportion of assignment of farm seeds from 2008 to the market seed cluster (i.e., cluster 1), compared to farm seeds from 1985.

Climate and barley yield

Several episodes of severe agricultural drought occurred during the study period, with the worst droughts occurring in 1990 and in 2002 (Fig.2). The highland site (Chefchaouenne) had higher drought indices than the lowland sites in all years, indicating that drought was likely to have had a more severe impact in the more marginal farmland situated inland and at higher elevations. In 2007, a particularly bad drought occurred, prompting nationwide days of prayer for rain (Ghanmi, 2007; Karam, 2012) (data for the scPDSI not available), and resulting in one of the four worst harvests in the past 52 years (FAOSTAT, 2010).

Discussion

This study shows a significant decrease of the genetic structure in the population of a traditional barley variety in northern Morocco over a 23-year period. Despite the changes in genetic structure, overall diversity parameters for the populations in 2008 remain similar to those for 1985 and therefore the large decrease in genetic structure cannot be attributed to a decrease in the overall genetic diversity of these populations over the past 23 years. Furthermore, morphological comparisons in Taounate Province (Jensen et al., 2012b) indicated that the overall phenotype of the dominant traditional variety cultivated in the region (*Beldi*), has not changed in this time. Taken together, these results are an indication that farmers in this large and heterogeneous region have maintained the identity and diversity of their traditional variety despite concerns of possible genetic erosion as a result of the introduction and increasing availability of modern varieties (FAO, 2009). This is consistent with results of survey data where farmers in Morocco have reported a preference for traditional varieties, citing adaptation to local conditions as one of the reasons for this preference (Jarvis et al., 2008; Jensen et al., 2012b).

In theoretical models of crop metapopulations, genetic structure (Fst) is predicted to decrease with increasing migration frequency and/or increasing extinction events (Slatkin, 1987; van Heerwaarden et al., 2009). Therefore, the observed decrease in structure for *Beldi* could be linked to changes in gene flow among populations or between local markets and populations. It could also be linked to increased extinction events of local populations, causing farmers to replenish their seed supply frequently from a more genetically homogenous pool.

Two factors, infrastructure development and agricultural drought, could have contributed to both increased gene flow and increased local extinction over the time period of this study. Furthermore, these two factors have the potential to interact to increase both the frequency of gene flow and the distance over which gene flow occurs.

Historically, in Morocco, poor roads that are subject to frequent closures due to flooding and poor maintenance have isolated all regions outside of the major city centers. Travel under such conditions is both difficult and costly, and the majority of trade occurs in periodic market places that convene once a week and attract vendors and buyers from a small radius of approximately 20-30 kilometers (Mikesell, 1958). Under these conditions, seed movement is expected to be extremely limited over large geographical distances. However, recent development initiatives by the Moroccan government have prioritized the improvement of roads in rural areas and there is increased market access for people living in remote areas, with access to a good quality road decreasing the time required to access a market by 50% (World Bank, 1995). In 1995, the Moroccan government implemented the National Rural Roads Program 1 (NRRP1), a 10-year program that resulted in the construction or improvement of 11 240 km of roads in rural areas, to improve the access of rural populations to markets, education and other services (African Development Bank, 2007). A subsequent project (NRRP2), implemented from 2005-2015 will result in the construction of an additional 15 560 km of rural roads (World Bank, 2011). Together, these projects have increased road accessibility in the country from 33% in 1995, to 54% in 2005 and 70% in 2010, meaning that there is increased

market access for people living in remote areas (Buerli et al., 2008). Under these conditions, farmers can access markets much more easily and vendors may sell in several markets located at larger distances from one another, due to the decreased cost of travel (Ghosh, 1981). This could result in higher gene flow throughout the region and between populations that were previously isolated.

Although transport infrastructure can potentially facilitate gene flow by increasing access of farmers to seeds from a broader geographic region, it is ultimately the individual farmer who makes the choice to purchase or not to purchase seeds from markets in any given year. Seed purchase and exchange in local markets can have a positive effect of enhancing intravarietal diversity on-farm, helping farmers to access a high quality and diversity of varieties that are appropriate for their region (Salazar and Winters, 2012). However, this is dependent on both the quality of seed available in the market, and the quality of the information available regarding the seed (Lipper et al., 2012).

A previous study of seed exchange networks in Taounate province found that 70-90% of farmers use their own seed to sow the barley crop in a specific year, while the remaining 10-30% of farmers purchase seed from local markets (Arbaoui, 2003; Jensen et al., 2012b). While many farmers (25-65%, depending on the location) report never having purchased market seeds, many other farmers report renewing their seed stock periodically at intervals ranging from every year, to every five years or more. It is therefore likely that, over time, a large number of farmers will receive inputs of market seed into their seed stock. Therefore, if seed in markets originate from an increasingly large geographic area, it is likely that

the influx of market seeds could be responsible for decreasing the genetic structure of the on-farm barley population over time.

Change in drought patterns in the region could change the frequency at which farmers are obliged to purchase market seed. Drought causes fluctuations in crop yield and seed production and can cause crop failure (i.e., a local extinction event) that forces farmers to renew their seed supply in regional markets, a trend that is increasing in several seed exchange networks worldwide (Mahdi, 2002; Lipper et al., 2012). The most frequent reasons farmers in Taounate province cited for purchasing outside seed supplies was that their own seed stock was insufficient or that their own seeds were unviable (36% of respondents) (Jensen et al., 2012a). Both of these outcomes are the result of poor harvests in the preceding year, most likely as the result of a drought, although other possible causes include biotic stressors such as insect pests and fungal infection.

Droughts have been an increasing problem in Morocco since the beginning of the 20th century, and two episodes of drought-related rioting occurred in the 1980s (Swearingen, 1992). There are indications that this problem has intensified in the past decades. Prior to the first seed collection date in 1985 the Rif Mountains region experienced a long period with few meteorological droughts (1968-1979) (Till and Guiot, 1990) and many wet years with above average precipitation as reflected by positive PDSI (Palmer Drought Severity Index) values (Palmer, 1965; Nicault et al., 2008). The regional climate for the time period between the collection dates of 1985 and 2008 has been characterized by an increasing frequency and severity of droughts, with one in five years

characterized by drought prior to 1990, and one in two years characterized by meteorological drought since 1990 (Forster et al., 2004; Karrou, 2005), and an average PDSI value of -3.9 indicating the most severe droughts in the region since the 13th century (Esper et al., 2007). In particular, severe droughts in 1990, 1995 and a series of years with low scPDSI values from 1998-2002 are associated with dramatic decreases in barley production (FAOSTAT, 2010). Furthermore, ongoing climate change is predicted to cause continued warming and decreased precipitation in the region (Räisänen, 2005), thereby further increasing the number of droughts and increasing the frequency of crop failure. Under this scenario, it is likely that frequent and high levels of periodic gene flow from markets to farms will continue in the future, maintaining the absence of structure among these populations. Increased gene flow from markets to farms is supported by the more frequent assignment of seeds from 2008 than from 1985 to the dominant cluster present in markets.

There are potential limitations to using seed bank seed for this type of study that highlight the importance of maintaining samples for longitudinal conservation studies using careful sampling strategies and storage practices (Pennisi, 2011). In particular, institutions need to archive detailed information regarding the collection sites of traditional variety accessions to allow researchers and conservation biologists to select samples that are appropriate for their studies (de Carvalho et al., 2012). Although barley landrace accessions from Morocco are available for collection dates as early as 1923, information regarding their collection site was lost when samples were transferred between institutions, precluding them from inclusion in this study.

Overall, this study highlights the dynamic nature of *in situ* conservation and the possibility for cryptic changes in agro-biodiversity in the absence of long-term monitoring. We show that a traditional crop variety under farmer seed management has undergone a decrease in genetic structure in a region that has experienced rapid development, possibly due to increased gene flow among populations, compounded by increased drought frequency that has led to greater reliance on market seed. Although diversity remains high, and germplasm conserved on-farm remains distinct from the seeds available in the market, this indicates a vulnerability of crop varieties under traditional seed management to changes in market availability of germplasm and could potentially lead to a loss of local adaptation). In this situation, support for farmer-based seed multiplication and the conservation of a reserve of seeds in a community seed bank for distribution following drought years could help support on-farm conservation of traditional seed varieties (Beyene, 1997).

Tables

Table 1: Collection sites, site characteristics, and sample sizes for on-farm samples of *Beldi*.

Site code	Province	Sample Size		Elevation (m)	ACZ[a]	Site category
		1985	2008			
Field.1	Tetouan	30	12	14	SH-M-W	Coastal/River delta
Field.2	Tetouan	56	20	6	SH-M-W	Coastal/River delta
Field.3	Tetouan	18	18	455	SH-C-W	River valley
Field.4	Chefchaouen	20	21	1099	SH-C-W	Mountain
Field.5	Taounate	10	15	742	SH-C-W	Mountain
Field.6	Taounate	30	90	298	SA-M-W	River valley
Field.7	Taounate	20	30	308	SH-M-W	River valley

[a]ACZ: Agro-Climatic Zone as defined by the United Nations Food and Agriculture Organisation. The first letter denotes the moisture regime (SH=subhumid, SA=semi-arid), the second letter denotes the winter type (C=cool, M=mild) and the third letter denotes the summer type (W=warm) (FAO, 2006).

Table 2: Collection sites, site characteristics, and sample sizes for market samples of *Beldi*.

Site code	Province	Village	Market name[a]	Alt. (m)	N[b]	ACZ[c]	Site category
Mark.1	Chefchaouen	Centre Amssa	Jeudi Amssa	1	19	SH-M-W	Coastal/River delta
Mark.2	Chefchaouen	Ouad-Laou	Samedi Ouad-Laou	10	25	SH-M-W	Coastal/River delta
Mark.3	Chefchaouen	Bou Ahmed	Mardi Bou Ahmed	44	20	SH-M-W	Coastal/River delta
Mark.4	Chefchaouen	Bani Hassan	Mercredi Bani Hassan	606	25	SH-C-W	River valley
Mark.5	Chefchaouen	Centre Tanakoub	Mardi Tanakoub	578	21	SH-C-W	Mountain
Mark.6	Chefchaouen	Ville de Chefchaouen	Lundi de Chefchaouen	554	25	SH-C-W	Mountain
Mark.7	Chefchaouen	Centre Chrafat	Dimanche Chrafat	611	20	SH-C-W	Mountain
Mark.8	Chefchaouen	Centre Bab Taza	Mercredi Bab Taza	87	24	SH-C-W	Mountain
Mark.9	Chefchaouen	Centre Bab Barad	Lundi Bab Barad	1234	25	SH-C-W	Mountain
Mark.10	Taounate	Centre Khlalafa	Lundi Khlalafa	129	23	SH-C-W	Mountain
Mark.11	Taounate	Centre Ain Aicha	Dimanche Ain Aicha	248	25	SH-M-W	River valley
Mark.12	Taounate	Oualja	Lundi Oualja	104	28	SA-M-W	River valley

[a] First word of the market name indicates the day of the week that the market is held: Lundi=Monday, Mardi=Tuesday, Mercredi=Wednesday, Jeudi=Thursday, Samedi=Saturday, Dimanche=Sunday

[b] N: Sample size.

[c] ACZ: Agro-Climatic Zone as defined by the United Nations Food and Agriculture Organisation. The first letter denotes the moisture regime (SH=subhumid, SA=semi-arid), the second letter denotes the winter type (C=cool, M=mild) and the third letter denotes the summer type (W=warm) (FAO, 2006).

Table 3: Gene diversity, number of alleles, allelic richness and private allelic richness of *Beldi* in the sampled farmer fields and markets. Values are averaged over the SSR loci tested.

Year	Site code	No. alleles/locus	No. alleles/locus SD	Gene diversity	Gene diversity SD	Allelic richness[a]	Private allelic richness[a]
1985	Field.1	4.83	1.57	0.37	0.23	3.73	0.16
1985	Field.2	5.50	1.89	0.52	0.30	4.01	0.12
1985	Field.3	4.50	1.71	0.42	0.26	3.73	0.18
1985	Field.4	5.00	0.58	0.50	0.30	3.94	0.25
1985	Field.5	3.67	1.25	0.36	0.23	3.45	0.15
1985	Field.6	5.67	1.80	0.37	0.23	4.00	0.58
1985	Field.7	4.33	1.70	0.41	0.25	3.65	0.16
Mean		**4.79**	**1.50**	**0.42**	**0.07**	**3.79 (0.21)**	**0.23 (0.16)**
2008	Field.1	3.17	1.34	0.40	0.26	3.02	0.06
2008	Field.2	4.33	1.25	0.38	0.24	3.73	0.46
2008	Field.3	3.67	0.94	0.25	0.17	3.28	0.11
2008	Field.4	4.83	1.34	0.37	0.23	4.05	0.14
2008	Field.5	4.17	0.90	0.35	0.22	3.49	0.39
2008	Field.6	6.83	2.48	0.52	0.30	4.17	0.21
2008	Field.7	5.83	1.68	0.48	0.28	4.05	0.30
Mean		**4.69**	**1.42**	**0.39**	**0.09**	**3.68 (0.44)**	**0.24 (0.15)**
2009	Mark.1	3.83	1.46	0.42	0.26	3.11	0.05
2009	Mark.2	3.83	1.77	0.38	0.23	3.04	0.11
2009	Mark.3	4.00	1.53	0.40	0.25	3.08	0.11

Year	Site code	No. alleles/locus	No. alleles/locus SD	Gene diversity	Gene diversity SD	Allelic richness[a]	Private allelic richness[a]
2009	Mark.4	4.50	1.89	0.38	0.23	3.39	0.08
2009	Mark.5	4.00	1.53	0.42	0.26	3.03	0.09
2009	Mark.6	4.83	2.12	0.40	0.24	3.47	0.17
2009	Mark.7	4.00	1.53	0.35	0.22	3.24	0.24
2009	Mark.8	4.83	2.54	0.41	0.25	3.31	0.15
2009	Mark.9	3.33	1.49	0.37	0.23	2.73	0.02
2009	Mark.10	4.00	1.53	0.43	0.26	3.30	0.02
2009	Mark.11	4.50	2.14	0.38	0.23	3.45	0.16
2009	Mark.12	4.67	2.36	0.42	0.26	3.29	0.44
Mean		**4.19**	**1.82**	**0.40**	**0.02**	**3.20 (0.21)**	**0.14 (0.12)**

[a]Adjusted for sample size (using 10 as a reference) using rarefaction.

Table 4: Partitioning of genetic variation within and among populations for each year, based on AMOVA analysis.

Source of variation	**Year**	**Sample**	**d.f.**	**% variation**[a]
Among pops.	1985	Farm	6	4.93***
Among ind. within pops.	1985	Farm	177	92.20***
Within ind.	1985	Farm	184	2.87***
Among pops.	2008	Farm	6	-0.18 (NS)
Among ind. within pops.	2008	Farm	188	99.23***
Within ind.	2008	Farm	195	0.95***
Among pops.	2009	Market	11	1.94 (NS)
Among ind. within pops.	2009	Market	268	96.19***
Within ind.	2009	Market	280	1.88***

[a] ***: $p<0.001$, NS: Not Significant

Table 5: Pairwise Fst-values between farm sites for 1985 and 2008. Values above the diagonal are for 2008 and values below the diagonal are for 1985. Bold font indicates a significant value after applying a Bonferroni correction (i.e., $p<0.00238$).

Year		2008	2008	2008	2008	2008	2008	2008
	Site	Field.1	Field.2	Field.3	Field.4	Field.5	Field.6	Field.7
1985	Field.1		-0.069	0.008	0.023	0.078	-0.131	-0.049
1985	Field.2	-0.002		0.025	0.095	**0.158**	-0.016	0.032
1985	Field.3	**0.135**	**0.106**		0.101	0.119	-0.035	0.047
1985	Field.4	0.065	**0.070**	**0.112**		**0.149**	-0.001	0.026
1985	Field.5	**0.154**	**0.095**	0.066	**0.151**		**0.090**	**0.117**
1985	Field.6	0.056	0.029	**0.108**	0.005	**0.152**		0.016
1985	Field.7	**0.068**	0.051	**0.113**	0.063	**0.200**	0.055	

Table 6: Partitioning of genetic variation within and among different pairs of samples (1985, 2008 and market) based on AMOVA analysis.

Source of variation	**Groups**	**d.f.**	**% variation**[a]
Among groups	85 & 08	1	0.09 (NS)
Among pops. within groups	85 & 08	12	2.49***
Among ind. within pops.	85 & 08	365	95.52***
Within ind.	85 & 08	379	1.90***
Among groups	08 & market	1	11.50***
Among pops. within groups	08 & market	17	0.97**
Among ind. within pops.	08 & market	456	86.22***
Within ind.	08 & market	475	1.30***
Among groups	85 & market	1	11.95***
Among pops. within groups	85 & market	17	2.74***
Among ind. within pops.	85 & market	445	83.28***
Within ind.	85 & market	464	2.03***

[a] **: $p<0.01$; ***: $p<0.001$, NS: Not Significant

Table 7: Results of tests for isolation by distance in each year using the Mantel test.

Year	Sample type	N[a]	Correlation coefficient	R-squared[b]
1985	Farmer seed	7	-0.187	0.035 (NS)
2008	Farmer seed	7	-0.268	0.072 (NS)
2009	Market seed	12	0.375	0.141*

[a] N: Number of sites
[b] *: $p<0.05$, NS: Not Significant

Figures


Field.1
Field.2
Field.3
Field.4
Field.5
Field.7
Field.6
© 2012 Cnes/Spot Image
Data SIO, NOAA, U.S. Navy, NGA, GEBCO
63 km
34°53'42.49" N 5°07'45.95" W elev 671 m
Eye alt 266.98 km
Google earth


Mark.1
Mark.2
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Mark.11
Mark.12
© 2012 Cnes/Spot Image
Data SIO, NOAA, U.S. Navy, NGA, GEBCO
63 km
34°53'42.49" N 5°07'45.95" W elev 671 m
Eye alt 266.98 km
Google earth

Figure 1: Map of the study area and sampling locations with proportion of samples assigned to each of two clusters identified using STRUCTURE (cluster 1=blue, cluster 2=red). (a) Assignment to clusters for fields sampled in 1985 (by ICARDA) and re-sampled in 2008. Results for 1985 are in the chart situated to the left for each site and results for 2008 are in the chart situated to the right. (b) Assignment to clusters for seed sampled from markets.

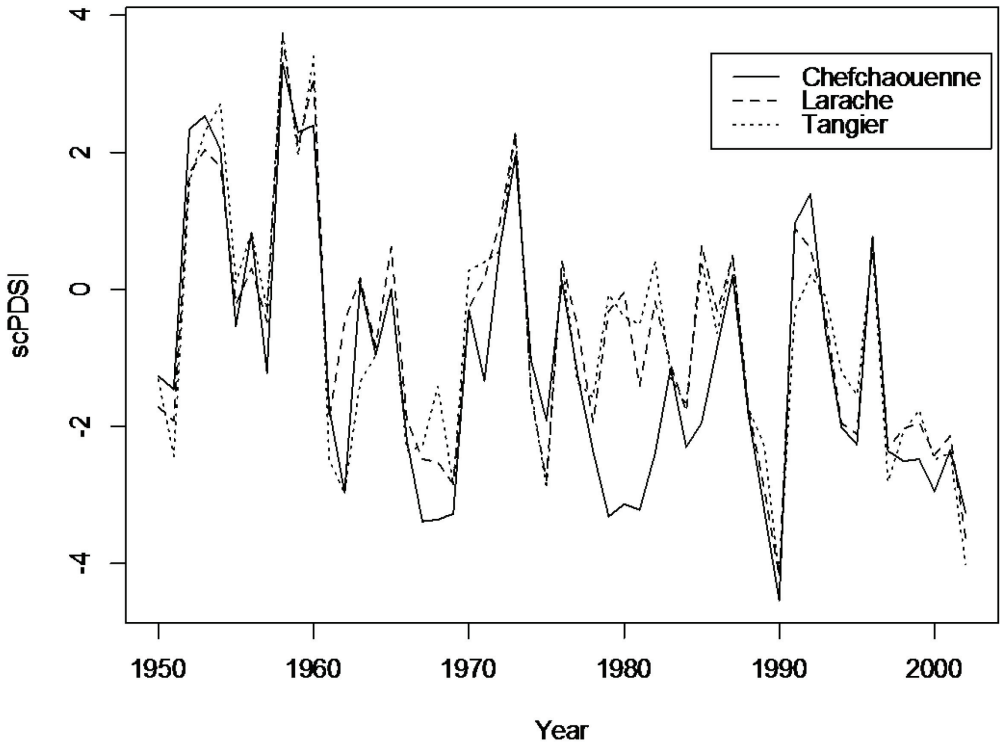

Figure 2: Drought severity for three sites in or near the study region as indicated by scPDSI values from 1950-2002. Chefchaouenne is an inland, mountainous region, Tangier is on the Mediterranean coast and Larache is on the Atlantic coast.

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Linking statement

In Chapter 3 I documented temporal changes in genetic structure in barley populations in Northern Morocco. I pointed out that these changes could impact the local adaptation for adaptive traits, in particular disease resistance. In Chapters 4 and 5, I pursue this line of investigation by investigating changes in resistance to a fungal pathogen of barley, powdery mildew (*Blumeria graminis* f.sp. *hordei*). In Chapter 4, I begin this work by identifying the virulence genes present in the pathogen population in the study region and characterising both spatial and temporal variation in the distribution of these virulence genes.

CHAPTER 4: High diversity, low spatial structure and rapid pathotype evolution in Moroccan populations of *Blumeria graminis* f.sp. *hordei*

Jensen HR, Dreiseitl A, Sadiki M, Schoen DJ. High diversity, low spatial structure and rapid pathotype evolution in Moroccan populations of *Blumeria graminis* f.sp. *hordei, European Journal of Plant Pathology*, doi: 10.1007/s10658-013-0166-y

Abstract

Limited information is available about the spatial distribution and evolution of *Blumeria graminis* f.sp. *hordei* populations in North African countries, such as Morocco. Frequencies of virulence alleles in *B. graminis* populations are mainly driven by selection exerted by host resistance genes in addition to neutral processes such as migration and genetic drift. In Morocco, in contrast to Europe, there has been no systematic deployment of resistant cultivars, although some R genes are present in the traditional varieties. This is expected to result in the evolution of pathotypes with virulence to different R genes, and higher diversity in Morocco compared to Europe. To test this, we used 24 differential cultivars to characterise 72 isolates from Morocco in 2009. We assessed diversity and spatial structure of pathotypes and compared them to past isolates from the same area (collected in 1992). There was a high diversity of pathotypes. Isolates from 2009 were distinct from isolates from 1992, due to loss of virulence to *Mla12*, increased virulence to *Mla8, Mla3* and *Mlk1,* and decreased virulence to *Mla6, Ml(Ru2), Mlg* and *MlLa.* Many virulences were different from those observed in European and Asian populations of *B. graminis.* At the spatial scale investigated, airborne dispersal and a lack of strong selection in the host population likely prevented the formation of population structure and allowed the accumulation of high isolate diversity. The evolution of novel and distinct pathotypes since 1992 is likely attributable to gene flow from Europe and selection by the host population in Morocco.

Abbreviations: *Bgh: Blumeria graminis* f.sp. *hordei*

Introduction

The fungal foliar pathogen *Blumeria graminis* (DC.) Golovin ex Speer f.sp. *hordei* Marchal (hereafter referred to as *Bgh*), causes powdery mildew on barley (*Hordeum vulgare* L. ssp. *vulgare*), resulting in yield losses worldwide. *Bgh* evolves virulence to new resistance genes (R genes) rapidly, and resistance based on specific major genes breaks down in as little as two to four years (Brown, 1994). Although *Bgh* evolution has been extensively studied in Europe, very little information is available for neighbouring countries in North Africa, such as Morocco.

Barley is a major crop in Morocco, occupying 2.2 million hectares and yielding 3.8 million metric tonnes annually (FAOSTAT, 2009). This represents 46% of the cereal cultivation area and 31% of the arable land surface (FAOSTAT, 2009). Powdery mildew caused by *Bgh* is an important disease of barley in North Africa with estimated yield losses ranging from 10 to 30% (Caddel and Wilcoxson, 1975). Both ecological and agricultural factors that influence the diversity and virulence of the pathogen are significantly different in Morocco compared to Europe, in particular the climate, the methods used to control fungal pathogens, and the genetic composition of the host barley population.

In contrast to Europe, the hot and dry summers in the Mediterranean climate of Morocco favour an obligate sexual stage with recombination (Wolfe et al., 1992). This is expected to generate a high diversity of genotypes and pathotypes (combinations of virulence genes) (Bousset and de Vallavieille-Pope, 2003b), thus providing raw material for natural selection. Another contrast between the regions concerns fungicide use, which is much more common in

Europe than in Morocco. The use of systemic fungicides is expected to lead to selection for fungicide resistance in *Bgh*, which is usually conferred by a mutation at a single locus (Brown et al., 1996). This, in turn, may cause selective sweeps reducing diversity at linked loci, which could include one or more virulence loci. The absence of fungicide use in Morocco removes this particular selection pressure and might therefore be expected to favour increased genotype and pathotype diversity in the *Bgh* population.

A third important factor that can influence *Bgh* evolution is the genetic composition of the host barley population, particularly with respect to R genes (Andrivon and de Vallavieille-Pope, 1993). In Europe, the host population of barley is generally composed of genetically homogeneous cultivars and the same R-gene combinations are deployed over large areas. This causes strong selection for *Bgh* isolates with virulence to these R genes and rapid evolution of new, adapted pathotypes. In Morocco, 85% of the barley cultivation area is occupied by traditional varieties that are heterogeneous for R genes (Jarvis et al., 2008; Jensen et al., 2012), with the remaining 15% devoted to more homogeneous modern cultivars (Saidi et al., 2006). The traditional varieties have varying frequencies and combinations of many different R genes, the most commonly identified of which are: *Mla8, Mlat, Ml(Ch)*, *Mla3, Mla6, Mla14, Mlg, Ml(CP*) and *Mla22* (Czembor and Czembor, 2000b; Czembor, 2001; Jensen et al., 2012). In Morocco, there has never been any formal, large-scale deployment of modern cultivars containing specific R genes or R-gene combinations (Saidi et al., 2007). Overall, the genetic heterogeneity of the host barley population in Morocco should result in weaker directional selection pressure on *Bgh* for virulence to specific R genes

than in Europe. This should lead to higher diversity of virulence genes and virulence gene combinations.

There is a strong spatial segregation between the traditional varieties and modern cultivars of barley in Morocco. Traditional varieties occupy most of the barley cultivation area on smallholder farms in mountainous regions and on marginal farmland (Moore et al., 1998; Ceccarelli et al., 2001). Modern cultivars are more common (although not ubiquitous) on larger farms in the fertile coastal regions.

It is difficult to predict how these combined factors will influence the diversity, spatial structure and evolution of pathotypes in the *Bgh* population in Morocco. On the one hand, increased recombination and decreased fungicide use could be expected to maintain a high diversity of pathotypes, which in turn would create the potential for rapid evolutionary change in the pathogen population. On the other hand, the genetic heterogeneity of the host population and the lack of systematic deployment of novel R-gene combinations could relax selection for novel virulence combinations and potentially slow the emergence of novel *Bgh* pathotypes compared to Europe. Within Morocco, the higher prevalence of homogeneous modern cultivars in coastal regions might lead to decreased pathotype diversity compared to inland regions. Different climates can also have an impact on fungal pathotypes (in the absence of different selection pressures in the host population) and this could lead to different evolutionary outcomes in different regions of Morocco, particularly between coastal and mountainous areas (Mboup et al., 2012).

The population genetics and virulence of the pathogen in Morocco have been studied only once, in 1992 (Yahyaoui et al., 1997). That study showed high virulence diversity and high virulence complexity, consistent with expectations for this region. However, there has been no analysis of the spatial distribution of the pathogen within Morocco; pathotypes could be expected to vary between regions if the composition of the barley population is variable and/or crop management processes are different between regions. Furthermore, we have no information regarding the evolutionary changes in pathotypes over time because data are only available for one reference year. Because Morocco is potentially exposed to gene flow from *Bgh* populations in Europe and the R gene composition of the host barley population is also variable over time, it is expected that the virulences in the *Bgh* populations have also evolved in the intervening years.

Bgh in Morocco is a potential source of spores for neighbouring countries in both Europe and North Africa (Brown and Hovmoller, 2002). As such, it is important to know how *Bgh* populations are structured geographically, how novel pathotypes are evolving and to what extent populations of *Bgh* in Morocco are divergent from those of other countries. Here we test the hypotheses that virulence frequencies differ among populations of *Bgh* in Morocco, that virulences and pathotypes have evolved during the 17-year period between samples in Morocco, and that pathotype diversity is higher in Morocco than in Europe. We assess the frequency of virulence to specific R genes, pathotype diversity, virulence complexity and spatial structure of *Bgh* isolates collected in Northern Morocco in 2009. We then compare these data to those for *Bgh* isolates from Morocco in 1992

to identify any evolutionary changes in virulence allele frequencies and pathotypes. We also compare our findings to similar studies from Tunisia, Europe, the Middle East, and China to identify larger temporal and spatial patterns in the evolution of *Bgh*.

Materials and Methods

Collection, multiplication, and testing of isolates

We collected *Bgh* isolates in Central and Northern Morocco in February of 2009 from five sites representing different agroecological regions of Central and Northern Morocco (Fig. 1). The sites are denoted as "Tao.1", "Tao.3", "Tao.4", "Tao.6" and "Rabat". Sites with the prefix "Tao." are located in Taounate Province in the Rif Mountains and the "Rabat" site is in the coastal region of Rabat-Salé-Zemmour-Zaër. Barley leaves with *Bgh* colonies were placed in Petri plates of water agar (5 g l^{-1}) containing 40 ppm of benzimidazole (Alfa Aesar, Lancaster, UK). We collected 20 isolate samples per site with a distance of at least 100 m between samples. All collections were done in fields of a traditional barley variety (referred to locally as *Beldi*). The plates containing barley leaves were placed in a growth chamber at 12:12 h L:D, light intensity of 17 µmol/m^2/s and a temperature of 18 +/- 1°C until sporulation.

Leaf segments (approximately 2.5 cm in length) were excised from the primary leaves of seedlings (GS=12) of the susceptible cultivar Rabat 071 (Wiberg, 1974) and placed on fresh Petri plates of benzimidazole agar. Fresh segments were then inoculated using a sterilized needle to transfer conidia from a

single *Bgh* lesion collected in the field. Plates were returned to the growth chamber for 8-9 days until the new lesions sporulated. This process was repeated two more times to obtain pure isolates. Subsequently, conidia from sporulating isolates were transferred to fresh leaves of Rabat 071 every 8-9 days. Due to isolate mortality during field collection, final sample numbers for the sites were: n=4 (Tao.1), n=19 (Tao.3), n=15 (Tao.4), n=15 (Tao.6) and n=19 (Rabat), for a total of 72 isolates.

The virulences present in *Bgh* isolates were determined in assays carried out from March 2009 to June 2009 in Rabat, Morocco. We used 22 near-isogenic lines (NILs) of the barley cultivar "Pallas" to differentiate virulences to different R genes (Kolster et al., 1986). We also used the cultivars Triumph and Lotta to differentiate between virulence to the R genes *Ml(Ab)* and *Mla7*. For brevity we refer to the combination of the NILs and the Triumph and Lotta cultivars as the "differential cultivars" (see Table 1 for details). Seedlings of the 24 differential cultivars and a Moroccan cultivar "Taffa" , that was resistant to all of the Moroccan isolates tested in a pilot study in 2008, were grown to the 2-leaf stage. Individual leaf segments excised from fully expanded primary leaves of the differential seedlings (GS=12) were placed in Petri plates containing benzimidazole agar. Each plate also contained a segment of the susceptible cultivar Rabat 071 and the resistant variety "Taffa" to act as controls for successful inoculation. Leaves were inoculated with individual *Bgh* isolates using a settling tower (100 mm by 250 mm) to distribute conidia at a density of approximately 8 per mm^2 (verified by counting under a microscope). Inoculated plates were incubated in the growth chamber for 8 days and reaction types of the

isolates on each differential were classified using a 0-4 scale with nine reaction categories (Torp et al., 1978) (Appendix II, Table S2). Only plates that showed a reaction type of 4 (fully susceptible) on the Rabat 071 leaf segment were used for reaction type readings, as a control for successful inoculation and pathogen development. In the rare cases where there was not a reaction type of 4 on Rabat 071, the inoculation was considered to have failed and was repeated. Isolates producing reaction types 0 through 3 were denoted as avirulent and those producing reaction types 3-4 and 4 were denoted as virulent because these latter reaction types indicate that the *Bgh* isolate was able to both infect and sporulate successfully. The test was repeated two times for each isolate to ensure accuracy. In the rare cases (less than 5%) where both inoculations gave different reaction types, a third inoculation was conducted and the more frequent reaction type was used. Reaction types were coded as binary data (1=virulence, 0=avirulence).

Data analysis

For each sampling site, we calculated the frequency of virulence to each differential as $p=x/n$, where x is the number of times a virulent reaction type was detected and n is the total number of samples tested. The standard error of each virulence frequency was estimated from the binomial variance. We tested for differences in the distribution of virulence allele frequencies among sites using a Chi-squared test. Because of the small number of surviving isolates from site "Tao.1" (n=4), it was excluded from this and all subsequent analyses, although we have reported the pathotypes from this site for reference purposes (Appendix II, Table S2).

The total number of virulent reaction types for each isolate was calculated and reported as the virulence complexity. The frequency of each virulence complexity value was determined for each site. The frequencies of isolates with low complexity (3-6 virulence alleles), and of those with high complexity (10-12 virulence alleles) were pooled. The distribution of virulence complexity values among sites was evaluated using a Chi-squared test.

The compiled reaction type data for each isolate on the differential cultivars were coded as individual pathotypes using octal notation (Gilmour, 1973). We calculated the diversity of pathotypes within each site and for the entire region (all sites combined) using the normalized Shannon-Weaver index (Hw') to allow for comparison across sites with different sample sizes:

$$Hw^1 = -1/\ln(n) \times \sum_{i=1}^{m} p_i \ln(p_i)$$

where n is the sample size, p_i is the frequency of the ith pathotype and m is the number of pathotypes (Shannon and Weaver, 1963; Mekwatanakarn et al., 2000).

Comparison to *Bgh* populations from Morocco and Tunisia in 1992

To assess temporal changes in *Bgh* virulence in Morocco, we compared the isolates we collected to those collected in Morocco (N=38) in 1992 (Yahyaoui et al., 1997). We also included data for isolates collected in Tunisia in 1992, as representatives of another North African country with similar conditions to Morocco (Yahyaoui et al., 1997). The isolates from 1992 were characterized using similar methodology as our study. The principal differences between our

methods and the methods of Yahyaoui (1992) are that, while we only sampled isolates in field of traditional varieties, they sampled in fields of traditional varieties in addition to the varieties "Tissa", "Marzouga" and "Anaceur". Furthermore, they assessed reaction type on whole seedlings, rather than on detached leaves as in our assay. Overall, however, we believe the results from our two studies should be comparable because the virulence and avirulence of each isolate was determined using the same criteria and the same differential cultivars. It is possible, however, that their use of a larger number of varieties to collect isolates could result in higher diversity for the 1992 isolates compared to our study.

Although the spatial distribution of the Moroccan samples from 1992 is slightly different from the 2009 samples, we assumed that spatial distribution differences between the two collections are not important because of the lack of strong geographic structure in the Moroccan *Bgh* population (as described in the Results section). We excluded data for differential cultivars that were not available for both collection years (i.e., differentials P01, P07, P11, P19, Triumph and Lotta), resulting in the retention of 21 differentials for the analysis.

We calculated virulence frequencies and standard errors for the 1992 data from Morocco and compared these values to those for 2009 using a Chi-squared test. We compared virulence data from both years to the R genes present in the host barley populations in 1985 and 2008 (data from Jensen et al., 2012). To summarize population differentiation, we calculated pairwise Fst-values (Nei, 1973) among sites and between years for isolates collected from Morocco in 1992 and 2009. We used FSTAT for Windows, V 2.9.3.2 (Goudet, 2002) for Fst

calculations. Significance of Fst values was determined after applying a Bonferroni correction for multiple comparisons.

Comparison to *Bgh* populations from Europe and Asia

To compare the diversity of *Bgh* in Morocco and Europe, we calculated the normalized Shannon-Weaver diversity index for the isolates reported in this study and for isolate samples collected in France in 1989 (Andrivon and de Vallavieille-Pope, 1993) and in Morocco in 1992 (Yahyaoui et al., 1997). We also included previously unpublished virulence data for 150 isolates collected from the Czech Republic (Moravia region) in 2009, the same year we collected our isolates. The samples from France were collected by sampling airborne inoculum using a jet spore sampler containing fresh leaf segments of the susceptible cultivars "Igri", "Manchuria", or the breeding lines SM-1414 and HJ, laid in petri dishes of benzimidazole agar. The Moravian isolates were also obtained using a jet spore sampler containing fresh leaf segments of the susceptible cultivars "Pallas" and "B-3213" (Dreiseitl et al., 2006). This sampling stragegy means that spores are sampled from all the barley varieties growing in the region, rather than from one specific variety, and could potentially result in the sampling of a higher diversity of the isolates compared to our study, where isolates were sampled from only one variety. To ensure that values of the normalized Shannon-Weaver index were comparable between the different populations, we calculated the diversity index using a common number of differentials for each comparison. We used 12 differentials for comparisons to the data from France, 22 differentials for

comparisons to the 1992 data from Morocco, and 7 differentials for comparisons to the 2009 data from Moravia.

To situate the *Bgh* population observed in Morocco in 2009 within a broader spatial and temporal context, we used previously published virulence data from *Bgh* population samples collected worldwide between 1987 and 2009. We compiled data for virulence frequencies from France in 1987-1990 (Andrivon & de Vallavieille-Pope, 1993), Tunisia in 1992 (Yahyaoui et al., 1997), Israel and the Czech Republic in 1997 and 1999 (Dreiseitl et al., 2006), East China in 2003-2004 (Dreiseitl and Wang, 2007), the Czech Republic (Moravia) in 2009, and the winter barley growing region of China in 2006 (Zhu et al., 2012). We assembled the compiled data in a heat map displaying the frequency of virulence to each R gene in each population and year. It is possible to compare the results of these different studies because the genetic materials of the differentials used are the same in all the datasets (although not all differentials are common to all datasets). However, because assays were conducted at different times, and with some variation in sampling and assay conditions, the results of the comparisons must be interpreted with some caution.

Results

Bgh population in Morocco (2009): basic parameters and spatial structure

The Moroccan isolates from 2009 were virulent to 16 of the 24 differentials (Table 1). All isolates were virulent to *Mla8* (Pallas). There was also a high frequency of virulence (>70%) to: *Mlk1*; *Mlat*; *Mlh*; *Mlra*; and *Mla10* + *MlaDu2*. In contrast, none of the isolates was virulent to the resistance genes (or

gene combinations) of: *Mla7 + Mlk1 + MlaNo3; Mla7 + MlaNo3; Mla7 + MlaMu2; Mla9 + Mlk1; Mla9; Mla12 + MlaEm2;* or *mlo5*. Although some virulence frequencies appeared to differ among sites (*Mla3*, *Mla22*, *MlLa, Mlra*, *Mlnn*, *Mlg +Ml(CP)*), these differences were not significant in Chi-squared analyses, possibly due to insufficient power (Fig. 2).

Out of the 72 isolates tested, there were 61 unique pathotypes (Online Resource 2). As a result, pathotype diversity as calculated using the normalized Shannon index was extremely high both across the entire region sampled (Hw′=0.89) and within each of the four sites analyzed (Tao.3: Hw′=0.98, Tao.4: Hw′=0.93, Tao.6: Hw′=1.0 and Rabat: Hw′=0.98). All of the sites had at least one pathotype in common with one or more of the other sites, indicating that there may not be strong structure among sites with regards to the distribution of specific pathotypes within versus among sites.

Virulence complexity of the isolates ranged from 3-12 but was high overall, with a mean value of 8.2 (+/-1.7) virulent reactions per isolate. There was a marginally significant difference in the frequency of virulence complexity among sites (χ^2=22.9, df=12, p<0.05) (Table 2). Site Tao.6, in particular, had a much higher frequency (70%) of isolates with low virulence complexity (3-6 virulence alleles) compared to the other sites. Conversely, sites Tao.3 and Tao.4 had high frequencies (40%) of isolates with high virulence complexity (10-12 virulence alleles).

Comparison to *Bgh* populations from Morocco and Tunisia in 1992

In Morocco, some virulence alleles changed in frequency between 1992 and 2009. Frequencies of virulence to *Mla6 + Mla14*; *Mla12 + MlaEm2*; *Ml(Ru2)*; *Mlg + Ml(CP)* and *MlLa* decreased significantly between 1992 and 2009 (Table 1). Virulence to *Mla12 + MlaEm2* disappeared completely. Conversely, the frequencies of virulences to *Mla8*, *Mla3* and *Mlk1* increased by 20%, 33% and 16%, respectively, and virulence to *Mla8* became fixed in the population (100%). In some cases, changes in virulence frequency paralleled the presence or absence of R genes in the Moroccan host population of traditional barley varieties in particular in the case of *Ml(Ru2)*, where the frequency of virulence decreased significantly in parallel with the disappearance of the R gene from the barley population. For *Mla8* and *Mlat*, virulence in the pathogen population and resistance in the host have remained consistently high (Table 1). The frequencies of many virulence alleles remained unchanged between 1992 and 2009, with virulence *to Mla10 + MlaDu2; Mlra; Mlnn; Mlat* and *Mlh* remaining high and virulence to *Mla22* and *Mla23* remaining intermediate. Virulences to *Mla7* and *Mla9* were rare in 1992 and nonexistent in 2009. A small number of isolates virulent to *mlo5* were reported in 1992, but this virulence was not detected in 2009.

The Fst values among Moroccan sites in 2009 were low (0.001-0.041), and not significant, indicating low spatial structure (Table 3). However, Fst values between 1992 isolates and 2009 isolates from Morocco were much higher and significant, indicating that marked changes have occurred in the *Bgh* population in Morocco since 1992. Isolates from Tunisia had significant Fst-values with all

other groups of isolates, indicating differentiation among the North African populations of *Bgh*.

Comparison to *Bgh* populations from Europe and Asia

The diversity of *Bgh* populations in Morocco was extremely high in both 1992 and 2009, particularly when compared to *Bgh* populations from Moravia in 2009 and, to a lesser extent, France from 1987-1990 (Table 4). Comparison to isolates from Moravia (Czech Republic) collected in 2009 (i.e., contemporary to our samples) showed strong divergence (Fig. 3). Virulences to *Mla3* and *Mlk* were much higher in Morocco, whereas virulences to *Mla6, Mla12, MlRu2, Mlg* and *MlLa* were much higher in Moravia. There are strong regional trends in the virulence frequencies observed in isolates from Morocco, China, Israel, and France. In particular, virulences to *Mla7* + *MlaNo3* and *Mla9* are virtually absent in Morocco and East China, but occur at high frequencies in France, Israel and Moravia. Virulence to *Mlat* is nearly absent in China, but occurs at high frequencies in all other regions. Virulence to *Mla3* is much higher in Israel than in any other region. Virulence to *Mlra* is common in all regions.

Discussion

Bgh isolates collected from Northern Morocco in 2009 had many virulence alleles, high diversity and high virulence complexity, similar to earlier collections from Morocco in 1992. We found no detectable spatial structure in 2009 for virulence allele frequencies or pathotype distribution, and only marginal structure for virulence complexity. We did, however, identify a number of temporal changes in virulence allele frequencies between 1992 and 2009 that

reveal a distinct evolutionary trajectory of *Bgh* in Morocco compared to neighbouring European populations.

The diversity of isolate pathotypes in the Moroccan samples in 2009 is much higher than for the *Bgh* isolates from Moravia (Czech Republic) in 2009 and is also higher than for *Bgh* in France in 1989 (Andrivon and de Vallavieille-Pope, 1993). The high diversity that we report is, however, similar to the diversity of *Bgh* in Morocco and Tunisia in 1992 (Yahyaoui et al., 1997). The high diversity is likely connected to the genetic heterogeneity of R genes in the host population. The traditional barley varieties have few R genes per individual, but considerable variation in the identity of R genes among individuals. This results in the absence of strong directional selection for specific virulence alleles which, in turn, results in a high diversity of *Bgh* pathotypes (Bousset & de Vallavieille-Pope, 2003a). This is supported by data from East Germany and Denmark where crop cultivar mixtures (with a diversity of R genes) have been used as a disease-control strategy to avoid the "boom and bust" cycle associated with the cultivation of varieties with the same R genes. Fields planted to such mixtures had a high diversity of *Bgh* pathotypes (similar to our study) compared to neighbouring regions that used cultivar monocultures (Muller et al., 1996).

We did not identify any strong spatial structure for either virulence frequency or genetic differentiation at virulence loci in Morocco. This was unexpected because our samples cover a large area and encompass several sites with different agroecological characteristics. We had particularly expected to observe divergence between the mountain sites and the coastal site due to the wider use of traditional varieties in mountain regions and of modern cultivars in

coastal regions. The overall lack of spatial structure may be because there is no significant variation in the assortment of R genes present in the different sites, and therefore no difference in selection. In the absence of strong selection, it is likely that the ability of the pathogen spores to disperse over long distances (Limpert et al., 1999) together with periodic sexual reproduction results in a strong homogenizing effect on the population, and together these overwhelm stochastic factors (e.g., genetic drift) that could lead to differentiation among sites. The absence of divergence between mountain sites and the coastal site may also be due to the sampling strategy in which isolates were collected exclusively in fields planted to traditional varieties. It is possible that we might have identified stronger spatial structure had we sampled fields of modern cultivars in the coastal site, compared to fields of traditional varieties in the other sites.

There were variations in virulence complexity among sites. On a much larger scale of thousands of kilometers, complexity increases from West to East, consistent with the accumulation of virulence through selection as spores move on the prevailing winds and encounter new barley cultivars with different R genes (Limpert et al., 1999). Although the scale of our study is too small to expect to observe this trend, it is noteworthy that Rabat, the westernmost site, has a peak in complexity at 7 virulence genes, with decreasing numbers of isolates at higher levels of complexity compared to two of the sites further east (Tao.3 and Tao.4), that have few isolates with low complexity, and high numbers of isolates with high complexity of 10-12 virulence genes.

There have been significant changes (both increases and decreases) in the frequencies of specific virulence alleles in Morocco between 1992 and 2009.

These changes are mostly distinct from evolutionary trends in European populations of *Bgh.* Virulence to *Mla8* increased to fixation in Morocco between 1992 and 2009. By comparison, European *Bgh* populations have been fixed for *Mla8* virulence since at least 1992 (Muller et al., 1992; Hovmoller et al., 2000; Dreiseitl, 2004). The increased *Mla8* virulence in Morocco could be caused by gene flow from the European populations of *Bgh,* coupled with selection pressure for this virulence arising from the relatively high frequency of the *Mla8* R gene in traditional Moroccan barley varieties (approximately 60%) (Jensen et al., 2012).

Virulence to *Mla7* has remained virtually absent in Morocco since 1992, despite the high frequency of this virulence in neighboring Spain and other European countries where 100% of isolates are virulent (Molina-Cano et al., 1992; Limpert et al., 1999; Dreiseitl et al., 2006). This contrast is likely due to differences in R-gene deployment between Morocco and Spain (and Europe in general). There has not been any large-scale deployment of registered cultivars with the *Mla7* R gene in Morocco during the time period examined in this study (Kamal, 2008), and therefore no selection pressure for *Bgh* isolates with virulence to *Mla7.* Under these conditions, it appears that pathotypes can remain divergent over long periods of time despite low physical barriers to gene flow.

Virulence frequencies to *Mlg* and *Mla12* in Morocco also show differences from European trends. In Morocco, virulence to *Mlg* declined from 42% to less than 20% in 2009. By comparison, in Southern Spain, 100% of isolates were virulent to *Mlg* in 1992 (Molina-Cano et al., 1992) and 96% and 97% of isolates in the Czech Republic were virulent in 1997 and 1999, respectively (Dreiseitl et al., 2006). Similarly, the frequency of virulence to *Mla12*

was intermediate (47%) in Morocco in 1992 and decreased to zero in 2009 while it increased to 100% in Moravia (Czech Republic) in 2009. Although we do not have recent data regarding the presence of these R genes in the Moroccan barley germplasm, earlier reports have found them to be extremely rare (Czembor and Czembor, 2000a; Czembor and Czembor, 2000b), suggesting that this virulence may have declined in the absence of selection, perhaps due to a neutral process such as drift.

The presence of a small number of isolates in Morocco with virulence to *mlo* in 1992 was a potential source of concern because this is the most durable R gene identified to date (Lyngkjaer et al., 2000). If isolates with virulence to *mlo* arise in Morocco they could potentially spread to other regions where *mlo* is an important resistance gene. However, in 2009 we did not detect any virulence to *mlo*, suggesting that Morocco is not a risk as a source of *mlo* virulence at the present time. Also of note is the consistently high frequency of virulence to *Mlat* (90%), an R gene originally described from Moroccan germplasm and which has presumably been co-evolving with the pathogen for a long time. Virulence to *Mlat* is high throughout Europe, likely due to incorporation of *Mlat* in barley breeding efforts.

The Moroccan isolates shared more common trends in virulence/avirulence with isolates from East China than they did with isolates from Europe. There are some similarities in the host barley populations in both countries that could account for the similarities in the *Bgh* isolates present. As is the case in Morocco, a number of traditional barley varieties are grown in China, in addition to cultivars from the formal breeding sector. The traditional Chinese

varieties contain few R genes, mainly *Mla8, Ml(Ch)*, and *Ml(Bw)* (Dreiseitl and Yang, 2007). The Moroccan varieties also have few R genes and, interestingly, *Mla8* and *Ml(Ch)* are also among the most common. This should create a similar selection environment for the *Bgh* isolates. Furthermore, the absence and/or rarity of R genes such as *Mla7* and *Mla12* in both countries correlates with an absence or rarity of virulent isolates for these genes in Morocco in 2009 and in China (Dreiseitl and Wang, 2007). This is likely a case of convergent evolution, with similar selection environments in geographically distant regions producing similar populations.

Overall, we have identified trends in virulence evolution in Morocco, including isolates that are not yet fixed for virulences that are ubiquitous in Europe and a decrease in the frequency of some virulence alleles in 2009 compared to 1992. This indicates that evolution of *Bgh* in Morocco, while influenced by gene flow from European populations, has followed a divergent path. Increased recombination and decreased control of the pathogen in Morocco as well as gene flow from Europe may provide the raw genetic material for pathotype evolution. However, the parallels between the R genes present in the barley germplasm in Morocco and the virulence genes in the *Bgh* population suggest that selection pressure from the host is the defining element determining virulence in the Moroccan *Bgh* population, similar to cases observed in Europe. We did not note any strong effect of the site climate, although this question could be tested further with more sites specifically selected to represent a gradient of climatic conditions. The dominant effect of host population on isolate virulence is

further supported by the similarity between Moroccan and Chinese isolates evolving in genetically similar, yet geographically distant, selection environments.

Changes in agricultural policy and agricultural development in Morocco that affect the identity and/or the distribution of the R genes in the barley crop could potentially cause a rapid change in the evolutionary trajectory of *Bgh* in the region. In 2008, the Moroccan government implemented an ambitious agricultural development policy, ”Le Plan Maroc Vert” (trans: The Green Morocco Plan). This policy calls for the use of improved crop cultivars, increased agricultural efficiency and improved access of small farmers to agricultural technology and irrigation. The deployment of improved barley cultivars that rely on R genes that have not previously been used in the region could rapidly select for higher frequencies of virulence alleles that are already present at low frequencies in the population. *Bgh* spores from Spain could also provide a source of virulence for novel R genes. It will be important to take these factors into account when deploying new barley cultivars, particularly in the mountainous and marginal regions where small farmers have long been cultivating traditional varieties. Because of the general movement of *Bgh* virulence alleles from West to East (via aerially dispersed spores), changes in *Bgh* virulence evolution in Morocco can also affect countries further east in North Africa, as well as in Europe.

Tables

Table 1. Virulence frequencies (%) of *Blumeria graminis* f.sp. *hordei* isolates collected in Morocco in 1992 and 2009 to the differential cultivars and their associated R genes.

Differential	**R genes**	**Virulence frequency (SE)[a]**		**p-value[b]**	**R gene present in the host population[c]**	
		1992	**2009**		**1985**	**2008**
Pallas	*Mla8*	**79.0** (6.7)	**100** (0)	p<0.001	Yes	Yes
P02	*Mla3*	**31.6** (7.6)	**64.8** (5.8)	p=0.001	--	--
P03	*Mla6 + Mla14*	**68.5** (7.6)	**10.3** (3.7)	p<0.001	No	Very rare
P04A	*Mla7 + Mlk1 + MlaNo3*	0	0	--	--	--
P04B	*Mla7 + MlaNo3*	0	0	--	--	--
P06	*Mla7 + MlaMu2*	2.7 (2.6)	0	--	--	--
P07[d]	*Mla9 + Mlk1*	5.3 (3.7)	0	--	--	--
P08A[d]	*Mla9 + Mlk1*	7.9 (4.4)	0	--	--	--
P08B	*Mla9*	0	0	--	--	--
P09	*Mla10 + MlaDu2*	76.4 (6.9)	76.5 (5.2)	NS	--	--
P10	*Mla12 + MlaEm2*	**47.4** (8.1)	**0**	p<0.001	--	--
P12	*Mla22*	39.5 (8.0)	26.5 (5.4)	NS	--	--
P13	*Mla23*	21.1 (6.7)	10.3 (3.7)	NS	--	--

Differential	R genes	Virulence frequency (SE)[a]		p-value[b]	R gene present in the host population[c]	
		1992	2009		1985	2008
P14	*Mlra*	79 (6.7)	72.1 (5.5)	NS	--	--
P15	*Ml(Ru2)*	**50** (8.2)	**22.1** (5.1)	p=0.003	Yes	No
P17	*Mlk1*	**73.7** (7.2)	**89.8** (3.7)	p=0.031	--	--
P18	*Mlnn*	73.7 (7.2)	69.2 (5.7)	NS	--	--
P20	*Mlat*	94.8 (3.7)	91.2 (3.5)	NS	Yes	Yes
P21	*Mlg + Ml(CP)*	**42.2** (8.1)	**17.7** (4.7)	p=0.006	--	--
P22	*mlo5*	7.9 (4.4)	0	--	--	--
P23	*MlLa*	**60.6** (8.0)	**17.7** (4.7)	p<0.001	--	--
P24	*Mlh*	94.8 (3.7)	95.6 (2.5)	--	No	Very rare
Lotta	*Ml(Ab)*	--	50.0 (6.1)	--	--	--
Triumph	*Mla7 + Ml(Ab) + MlaTr3*	--	1.5 (1.5)	--	--	--

[a] Virulence frequency (and standard error) to the specific R gene(s). Values in bold are significantly different between years in the Chi-squared analysis. Data for 1992 are from Yahyaoui et al. (1997).

[b]Empty cells are cases where Chi-squared analysis could not be performed.

[c] Refers to the presence of the corresponding R gene in the barley germplasm from Morocco in 1985 and 2008 (data from Jensen et al., 2012). Empty cells are R genes for which the germplasm was not tested.

[d]These differentials have the same R genes originating from different genetic backgrounds.

Table 2: Distribution of virulence complexity of *Blumeria graminis* f.sp. *hordei* isolates among sites in Morocco in 2009 (χ^2=22.9, df=12, p<0.05).

	Proportion of each complexity class in each population (%)				
Site	**3 to 6**	**7**	**8**	**9**	**10 to 12**
Tao.3	0	18	35	33	40
Tao.4	10	27	15	17	40
Tao.6	70	18	15	17	7
Rabat	20	36	35	33	13

Table 3. Matrix of pairwise Fst values comparing *Blumeria graminis* f.sp. *hordei* populations from four sample sites in Morocco in 2009 and from Morocco and Tunisia in 1992. Values in bold are significantly different between sites after a Bonferroni correction.

Site and Year	**Tun (92)**	**Mor (92)**	**Mor, Rab (09)**	**Mor, Tao.3 (09)**	**Mor, Tao.4 (09)**	**Mor, Tao.6 (09)**
Tun (92)	0					
Mor (92)	**0.084**	0				
Mor, Rabat (09)	**0.263**	**0.165**	0			
Mor, Tao.3 (09)	**0.221**	**0.122**	0.016	0		
Mor, Tao.4 (09)	**0.247**	**0.149**	0.041	0.001	0	
Mor, Tao.6 (09)	**0.252**	**0.174**	0.018	0.023	0.029	0

Table 4. Diversity of *Bgh* isolates in Morocco, Moravia, and France, as measured using the normalized Shannon-Weaver diversity index for 7, 12 or 22 differentials (diff.).

			Normalized Shannon-Weaver index		
Region	**Year**	**n**	**7 diff.**	**12 diff.**	**22 diff.**
Morocco	2009	72	0.76	0.87	0.90
Morocco[a]	1992	38	0.78	0.94	1.00
Moravia (Czech Republic)	2009	150	0.49	--	--
East France[b]	1989	50	--	0.77	--
North France[b]	1989	91	--	0.79	--
Paris area[b]	1989	62	--	0.71	--

[a]Data from Yahyaoui et al., 1997.

[b]Data from Andrivon and de Vallavieille-Pope, 1993.

Figures

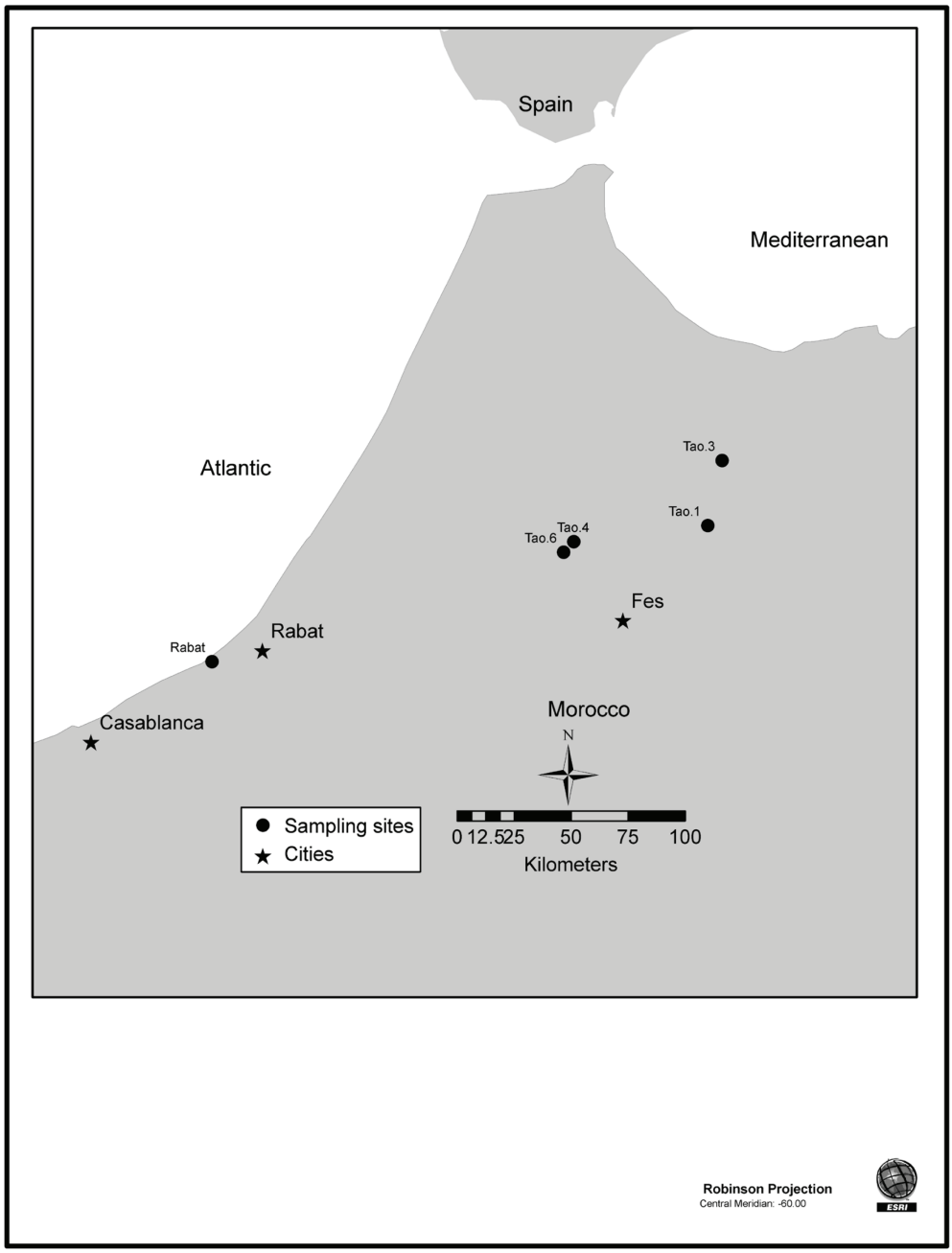
Spain
Mediterranean
Atlantic
Tao.3
Tao.1
Tao.4
Tao.6
Fes
Rabat
Rabat
Casablanca
Morocco
N
Sampling sites
Cities
0 12.525 50 75 100
Kilometers
Robinson Projection
Central Meridian: -60.00
ESRI

Figure 1. Sites in Morocco where *Blumeria graminis* f.sp. *hordei* isolates were sampled in February 2009. Site agro-climatic zones are defined according to the system of the United Nations Food and Agriculture Organisation where the first two letters denote the moisture regime (SH=subhumid, SA=semi-arid), the second letter denotes the winter type (C=cool, M=mild) and the third letter denotes the summer type (W=warm) (FAO, 2006). Site Tao.1 is SH-M-W, Tao.3 is zone SH-C-W, sites Tao.4, and Tao.6 are zone SA-M-W and Rabat is zone SH-W-W. The lowest elevation site (Rabat) is located at 23m and the highest elevation site (Tao.3) is located at 796m.

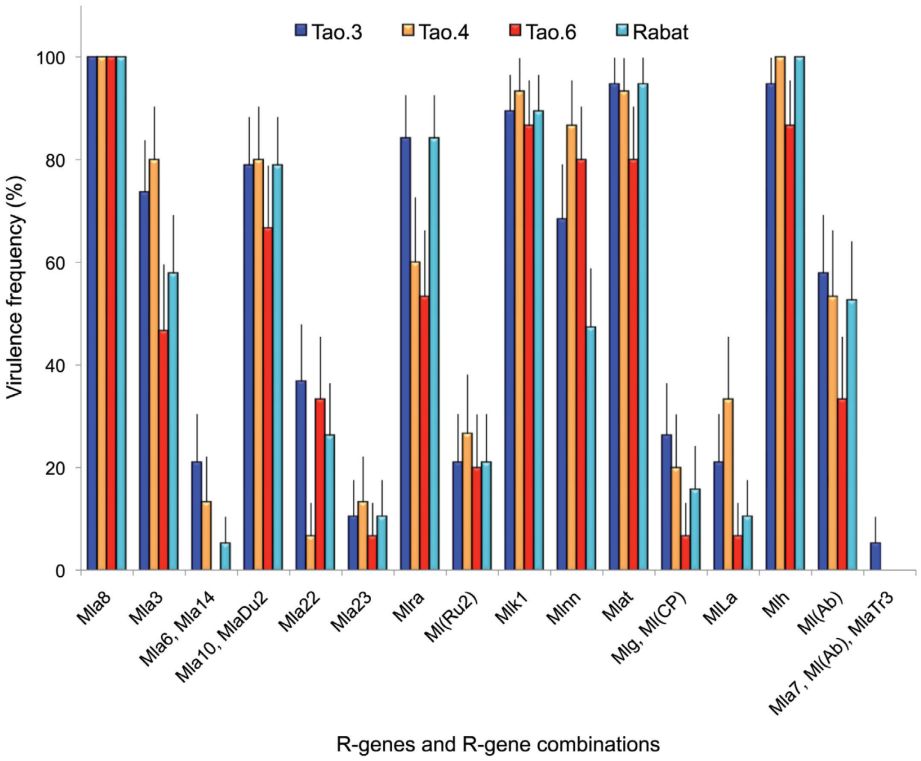

Figure 2. Virulence frequencies (+SE) in each site (in 2009) of *Blumeria graminis* f.sp. *hordei* isolates to the R genes and R gene combinations in the differential cultivars. There was no virulence to *Mla7 + Mlk1 + MlaNo3; Mla7 + MlaNo3; Mla7 + MlaMu2; Mla9 +Mlk1; Mla9; Mla12 + MlaEm2* or *mlo5* (data not shown). There were no significant differences in virulence frequencies among sites in Chi-squared analyses.

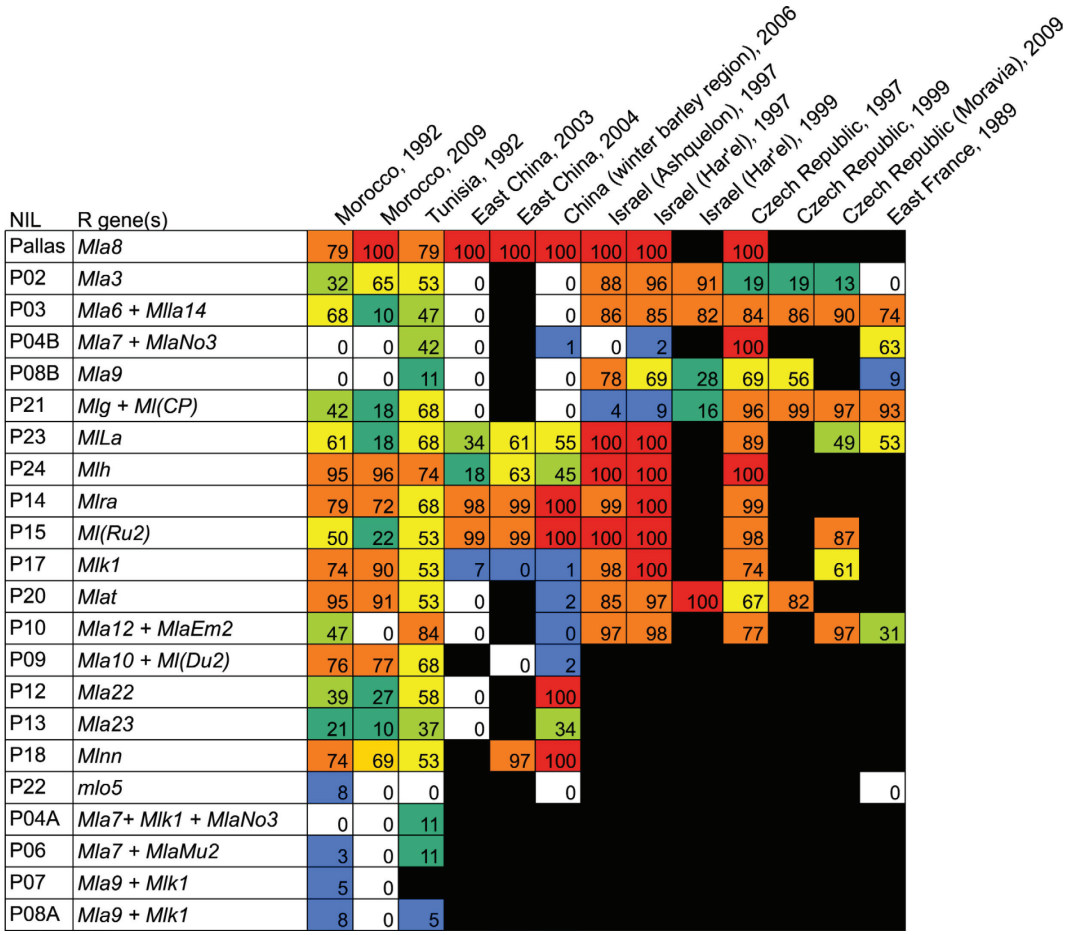

Figure 3. Heat map of virulence frequencies to R genes in a literature review of *Blumeria graminis* f.sp. *hordei* isolates in Europe, Asia, and North Africa from 1987-2009. Colours represent frequencies of virulence: red=fixation (100%); orange (70-99.9%), yellow (50-69.9%); light green (30.-49.9%); dark green (10-29.9%); blue (0.01-9.99%); white=absent (0); black=data unavailable.

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Linking statement

In Chapter 4, I demonstrated that *Blumeria graminis* f.sp. *hordei* has rapidly evolving combinations of virulence genes in northern Morocco. This means that *B. graminis* is a good model species to investigate whether new combinations of resistance genes evolve in local populations of barley as a result of selection pressure from a pathogen. According to many reports and policy recommendations, crop populations maintained on-farm should have higher resistance to local pathogens than populations maintained *ex situ*. However, this has never been explicitly tested. In Chapter 5, I test this question, again using seed material gathered in 1985 and maintained *ex situ,* (i.e., removed from the pathogen) compared to materials that have been maintained on-farm (*in situ*).

CHAPTER 5: The Red Queen and the seed bank: pathogen resistance of *ex situ* and *in situ* conserved barley.

Jensen HR, Dreiseitl A, Sadiki M, Schoen DJ 2012. The Red Queen and the seed bank: pathogen resistance of *ex situ* and *in situ* conserved barley. *Evolutionary Applications* **5**: 353–367.

Abstract

Plant geneticists have proposed that the dynamic conservation of crop plants in farm environments (*in situ* conservation) is complementary to static conservation in seed banks (*ex situ* conservation) because it may help to ensure adaptation to changing conditions. Here we test whether collections of a traditional variety of Moroccan barley (*Hordeum vulgare* ssp. *vulgare*) conserved *ex situ* showed differences in qualitative and quantitative resistance to the endemic fungal pathogen, *Blumeria graminis* f.sp. *hordei* compared to collections that were continuously cultivated *in situ.* In detached leaf assays for qualitative resistance, there were some significant differences between *in situ* and *ex situ*-conserved collections from the same localities. Some *ex situ* conserved collections showed lower resistance levels while others showed higher resistance levels than their *in situ* conserved counterparts. In field trials for quantitative resistance, similar results were observed, with the highest resistance observed *in situ*. Overall, this study identifies some cases where the Red Queen appears to drive the evolution of increased resistance *in situ.* However, *in situ* conservation does not always result in improved adaptation to pathogen virulence, suggesting a more complex evolutionary scenario, consistent with several published examples of plant-pathogen co-evolution in wild systems.

Introduction

In evolutionary biology, the Red Queen metaphor likens the process of evolutionary adaptation to a race in which the population (runner) must constantly move just to remain in the same place (Van Valen, 1973). This notion has become central in conservation biology as well, and a number of evolutionary studies have stressed the importance of continued adaptation in preventing extinction (Gomulkiewicz and Holt, 1995; Bell and Gonzalez, 2009). A related consideration is the concept of "lag load". Under changing environmental conditions, the mean phenotype of a population is expected to track, but lag behind, the shifting phenotypic optimum because of the time required for the population to respond to selection (Lynch and Lande, 1993). Lag load becomes a particular concern in the conservation of populations that are removed from their natural environment for a number of generations and may thus exhibit an even more pronounced lag in adaptation, because such isolation adds an additional period during which selection cannot track the phenotypic optimum (Schoen and Brown, 2001).

A specific and common example of a situation where removal from co-evolutionary interactions is expected to reduce population fitness occurs within the area of crop genetic resource conservation. Seeds from agricultural species ("crop germplasm") are typically collected and stored in institutional seed banks, a mode of conservation referred to as "*ex situ"* conservation. *Ex situ* conservation provides reserves of seeds for breeders, and/or for re-introducing varieties in the event of environmental or political catastrophes that lead to the loss of local crop germplasm (Clarke, 2003; Elina et al., 2005; Fowler, 2008). However, the isolation of crop germplasm from the environment, and the resulting lag load

could, in theory, result in significantly reduced fitness upon reintroduction**.** For this reason, *in situ* conservation, where crop germplasm is conserved on-farm, has been proposed as being an important complement to *ex situ* conservation because it is assumed to maintain the processes of co-evolution and adaptation of plant populations to changing biotic and abiotic conditions in a way that is not possible in a seed bank (Frankel, 1974; United Nations, 1992; Hamilton, 1994; Secretariat of the Convention on Biological Diversity, 2010)**.**

In recent years, a number of studies have highlighted the importance of considering an evolutionary framework both in the study and conservation of crop germplasm (Thrall et al., 2010; Thrall et al., 2011). Issues including adaptation of crop germplasm to climate change (Mercer and Perales, 2010), the interaction between altitudinal gradients and local adaptation of maize (Mercer et al., 2008) and the potential for weeds and invasive species to evolve from domesticated plants (Ellstrand et al., 2010) have all been explored. Few studies, however, have explicitly tested the prediction that crop varieties conserved *in situ* are better adapted to current abiotic and biotic conditions compared to related *ex situ* collections. Exceptions include documented changes in the flowering date and drought stress tolerance of rice varieties in response to agricultural intensification (Tin et al., 2001), and changes in flowering date and morphological traits of Hopi maize varieties (Soleri and Smith, 1995). We are unaware of any studies that have tested for changes in plant adaptation to fungal pathogens under *ex situ* and *in situ* conservation. Maintaining adaptation of crop germplasm to fungal pathogens and emerging pathogen strains is a particular concern because fungal pathogens of

crops cause economically and socially important yield losses worldwide (Burdon and Thrall, 2008).

There are two broad categories of host-plant resistance to fungal pathogens: qualitative resistance (also referred to as "race-specific" or "gene-for-gene") and quantitative resistance (also referred to as "polygenic" or "partial"). Qualitative resistance determines the ability of a specific pathotype of the pathogen to infect a specific genotype of the host plant. When resistance is effective, there is no infection when plants are exposed to the pathogen. However, if resistance fails, infection typically results in a disease phenotype. Pathogens and hosts co-evolve on a gene-for-gene basis (Flor, 1956), with reciprocal selection of host resistance genes (R-genes) (Paillard et al., 2000a; Paillard et al., 2000b) and pathogen virulence genes (Wolfe et al., 1992). Novel virulence alleles that overcome host resistance increase pathogen fitness and may reach high frequencies in the pathogen population. There is, however, a fitness cost associated with virulence alleles that could select for their loss if a corresponding resistance disappears in the host population (Cruz et al., 2000; Burdon and Thrall, 2003; Tian et al., 2003), and this limits the accumulation of virulence genes in the pathogen. The number of R-genes in the host plant is limited by the genetic architecture of the host (Wei et al., 2000) and allelism (Wei et al., 2002). *De novo* mutations or gene flow can also introduce novel alleles into the host or pathogen population, and recombination can result in novel combinations of virulence to host R-genes (i.e., novel pathotypes) as well as novel combinations of host resistance to the pathogen. This may result in an "evolutionary arms race", and in

this context the maintenance of host plant resistance is expected to be dependent on continued selection by, and adaptation to, the evolving pathogen population.

The second category of resistance, quantitative resistance, involves several genes that limit the spread of infection once gene-for-gene resistance has been overcome by the pathogen. Both types of resistance are important, but quantitative resistance is considered more durable as it is not pathotype-specific but is effective against all pathotypes of a pathogen species (Chelkowski et al., 2003). The genes for qualitative resistance sometimes map to the same quantitative trait loci as those for quantitative resistance (Maroof et al.. 1994; Falak et al., 1999) suggesting that some of the same genes may be involved in both systems (Toojinda et al., 2000; Backes et al., 2003; Shtaya et al., 2006; Shen et al., 2007; Poland et al., 2011). However, the presence of high qualitative resistance does not necessarily imply high quantitative resistance to a specific isolate, and *vice versa* (Wenzel et al., 2001).

There are two possible and contrasting outcomes regarding qualitative resistance of host-plant collections maintained *ex situ* versus *in situ*. First, *ex situ* collections (which by virtue of storage as seed are therefore not exposed to the current virulence combinations in the pathogen population) may lack the R-genes that match new pathogen virulences that evolved after the germplasm was removed from the environment, and so may show decreased resistance compared to *in situ* populations. Second, genetic drift in the *in situ* host population (perhaps due to a population bottleneck associated with colonization or demographic instability) may lead to loss of R-genes, and so collections conserved *ex situ* may maintain resistance alleles that no longer present in the field population (and to

which the pathogen has lost the corresponding virulence). This could lead to the opposite result, namely increased resistance in *ex situ* populations compared to *in situ* populations.

Although quantitative resistance is not directly connected to R-genes, different pathotypes vary in their aggressiveness to the host. Therefore, in the case of quantitative resistance, recurring mass selection by farmers of the healthiest plants is expected to maintain the quantitative resistance of the *in situ* populations relative to *ex situ* populations.

Here we study the relationship between conservation strategy and host plant resistance to a pathogen. Our test system is a traditional variety of barley (*Hordeum vulgare* L. ssp. *vulgare,* hereafter referred to as *H. vulgare*) together with the causative agent of powdery mildew of barley, *Blumeria graminis* (DC.) Golovinex Speer f. sp. *hordei* Marchal (hereafter referred to as *Bgh*), in Northern Morocco. We used detached leaf (*in vitro*) assays and field trials to measure changes in the qualitative and quantitative *Bgh*-resistance barley germplasm conserved both *in situ* and *ex situ* in geographically-paired collections. We also characterized the R-genes present in both of the *ex situ* and *in situ* germplasm collections.

Materials and Methods

Seed collections

Seeds of the traditional barley variety "*Beldi",* collected from five separate locations in Morocco in 1985, denoted Tao.1, Tao.3, Tao.4, Tao.5 and Tao.6 (Fig. 1) and conserved *ex situ* at ICARDA (International Center for Agricultural

Research in the Dry Areas, Aleppo, Syria) were used as one set of materials for this study. The collection sites were located in Taounate province and spanned a range of elevations and climatic conditions. For the second set of materials we re-sampled seeds of the "*Beldi"* variety from the same five locations in 2008, 23 years after the original collection date. To do this we sampled seeds within a 3 km radius of each original sampling location. We collected at least five samples from each of four different fields within each site, in order to replicate the sampling strategy that was used to collect the initial samples in 1985 (Perrino et al., 1986).

Maternal effects and long storage may cause phenotypic differences between the progeny of seeds from seedbank collections compared to seeds collected in the field (Dreiseitl, 2007). To remove these effects, we multiplied seeds from the original sample materials of both collections in the McGill University Phytotron in 2008-2009 (N=200 i.e., 20 samples per site, per year). Seeds were disinfected with a 2.5% sodium hypochlorite solution and washed in sterile dH_2O to suppress possible fungal contamination. Seeds were planted in eight-inch pots and were randomly assigned to one of 20 blocks (4X5). Greenhouse conditions were 14:10 h L:D, 21:18ºC and 1000 µmol/m^2/s PAR at midday. Plants were fertilized weekly with 20:20:20 NPK solution beginning at anthesis (GS=61) (Zadoks et al., 1974) and continuing through the end of grain filling (GS=79). Seeds were then harvested from each individual plant and equal numbers of seeds from each plant were used in subsequent tests.

Verification of seed collections using morphology and microsatellites

The hypothesis we test is whether plant populations maintained by Moroccan farmers under field conditions in specific locations continued to evolve resistance to the resident powdery mildew pathogen populations, compared with the same ancestral populations that had been earlier stored *ex situ* in the seed bank (i.e., away from the pathogen) for 23 years. We were concerned that, during these 23 years, different varieties of barley could have been intentionally introduced on some farms, and replaced the original material. If that were the case, the comparison(s) in question would not be examining the evolution of resistance in the *in situ* versus *ex situ* host plant populations, but instead, the change in resistance brought about by the introduction of new germplasm. While the human-mediated flow of new germplasm from one site to another is frequently a component of *in situ* conservation occurring in traditional farming systems, our principle objective in this study was to compare the evolution of materials maintained *ex situ* with those same materials maintained under field conditions in specific locations. Hence, we felt it important to exclude from analysis any sample pairs in which there was evidence of human replacement of the *in situ* maintained material.

We therefore used both morphological and microsatellite data to compare each paired collection in order to identify evidence of replacement. We first compared six qualitative morphological traits used for describing barley varieties: row number, spike density, lemma awn, lemma awn barbs, glume/glume awn length and rachilla hair length (Murphy and Witcombe, 1986; IPGRI, 1994). Seeds collected from 1985 and 2008 had identical distinguishing qualitative

characteristics indicating that they belong to the "*Beldi"* variety. "*Beldi"* is a traditional, 6-row variety, with a white aleurone layer, lax spike density, lemma awns, barbs along the entire length of lemma awns, glume plus glume awns of equal length to the kernels, and long rachilla hairs (Appendix III, Table S1). In the 2008 collections, we did find a small proportion of plants (<1%) belonging to two other traditional varieties: "*Roumi"* (a 6-row variety distinguished by dense spikes and smooth awns), and "*Rouiza"* (a 2-row variety). These samples were excluded from this study.

We then compared collections using three quantitative morphological traits: spike length, seed length and number of triplets per spike. We compared the values of these traits between years using a one-way ANOVA. The means were not significantly different between years (Appendix III, Fig. S1). These data provide an additional indication that there have not been any significant changes in the identity of the *Beldi* variety between the two collection years.

Finally, we used six microsatellite markers to test for genetic divergence between years. We collected leaf tissue from ten plants per sample, per year. Total genomic DNA was extracted from the leaf tissue using the DNA plant mini kit (QIAGEN). Samples were characterized for the microsatellite markers Bmag0013, Bmag0321, Bmag0345, Bmac0316, Bmag0125 and EBmac0541 (Ramsay et al. 2000) conducting PCR with M13-tailed primers and a universal primer (M13 (-43) (Schuelke, 2000) labelled with IRDye700 or IRDye 800 (Li-Cor). Primer sequences and PCR conditions are provided as Supporting Information (Appendix III, Tables S2-S3). PCR products were separated using acrylamide gel electrophoresis on a Li-Cor sequencer. Allele sizes were

determined using SAGA software (Li-Cor). Genetic divergence (Fst) was calculated between pairs of samples taken from the same site, in different years, using Arlequin 3.1 software (Excoffier et al., 2005).

The Fst-values at several loci indicated that approximately 25% of the samples from 2008 differed significantly from those of the 1985 samples collected from the same sites. This observed divergence likely reflects high rates of human-mediated gene flow into the sites (e.g., arising from replacement of seed stocks by farmers). Thus, these samples were excluded from the remainder of the study because they likely were exposed to pathogen conditions that were not representative of those in the seed collection sites. The population pairs that we retained for this study had not significantly differentiated in genotype frequencies between years as measured by Fst (Appendix III,Table S4).

Overall, based on the combined comparisons of morphological and genotypic data, apart from the samples excluded, there was little evidence that new varieties had been introduced into the 2008 sites since the 1985 collection. This validates the use of these collections from different years to study changes in resistance over time in the resident populations.

Collection and pathotype determination of *Bgh* isolates

In the field, single lesions were removed from excised leaves and transported to the laboratory, where they were maintained on five percent agar containing 40 ppm of benzimidazole (Alfa Aesar, Lancaster, UK), a senescence inhibitor. Isolates were kept in a growth chamber at 12:12 hours L:D, light intensity of 17 $\mu mol/m^2/s$ and temperature 18 +/- 1°C. When sporulation

occurred, 2.5 cm leaf sections of the susceptible barley variety Rabat 071 (Wiberg, 1974) excised from seedlings (2-leaf stage) were inoculated by shaking spores from infected leaves on to the fresh leaves. When these lesions sporulated, single-spore isolates were placed on fresh leaves. This process was repeated twice to obtain monospore isolates. The culture was maintained by inoculating fresh leaves of Rabat 071 at every 8-9 days.

In February 2008 we collected *Bgh* isolates in the sites where barley samples had been collected. Because two of the barley collection sites (Tao.4 and Tao.5) are very close together, we considered them to represent only one collection site for *Bgh*, and therefore we collected two isolates from each of four sites (Tao.1, Tao.3, Tao.4 /Tao.5 and Tao.6), for a total of eight isolates of *Bgh* (Fig. 1). These *Bgh* isolates were collected for the purpose of testing the qualitative resistance of the traditional barley varieties to sympatric *Bgh* isolates using *in vitro* detached-leaf assays. The collection sites for these isolates were within 3 km of the collection sites for barley. We also sampled four isolates from the field plot in Rabat and an additional fifteen isolates in the surrounding region to examine the diversity of *Bgh* pathotypes causing disease in field conditions. These isolates were used as indicators of the virulence gene combinations (pathotypes) present in the field trial for quantitative resistance, and to compare with the isolates used in trials for qualitative resistance.

To determine the pathotypes of the isolates, we used detached leaf assays with a differential set of 24 barley varieties, including 22 Pallas near-isogenic barley lines (Kolster et al., 1986) as well as the barley varieties Lotta and Triumph (Table 1). The near-isogenic lines share the same genetic background (Pallas) but

vary for the presence of specific resistance genes (R-genes) or combinations of R-genes. If an isolate can successfully infect a Pallas line containing a specific R-gene, that isolate is considered to be virulent towards that specific R-gene, following the gene-for-gene concept. Conversely, failure to infect denotes avirulence to the R-gene. By compiling the virulence/avirulence of an isolate to the entire differential set, the pathotype is deduced.

We grew seedlings of the differential set in the greenhouse for 2 weeks until they reached the two-leaf stage. Individual plant pots were covered with cellophane bags during this time to prevent accidental inoculation by airborne *Bgh* spores (while still permitting air circulation). Primary leaf segments measuring approximately 2.5 cm were excised from seedlings and placed in Petri dishes of agar containing 40 ppm benzimidazole (a senescence inhibitor). A settling tower (10 cm by 25 cm) was used to inoculate Petri dishes with a single-spore *Bgh* isolate with an inoculation density of approximately 8 conidia mm^{-2} (verified visually). Petri dishes were then placed in the growth chamber at 12:12 hours L:D and temperature 18 +/- 1°C. Reaction types were scored after 8-9 days using the nine-point 0-4 scale (including intermediate types) of (Torp et al. 1978) (Appendix II, Table SI). All assays were replicated twice and contained a susceptible (Rabat 071 variety) and resistant (Taffa variety) control to verify the efficacy of inoculation (we obtained these varieties from the Institut National de Recherche Agronomique (INRA), Rabat, Morocco). In accordance with standard practice, reaction types 0 through 3 were classified as isolate avirulence (coded as "0"), while reaction types 3-4 and 4 were classified as isolate virulence (coded as

"1") (Dreiseitl and Wang, 2007). These data were transformed to octal notation, where binary data for triplets of differentials are transformed to a single number to produce a numeric designation (the pathotype) representing each combination of virulence and avirulence genes (Limpert and Muller, 1994).

To compare the virulence of the isolates found in the field site to isolates used for detached leaf assays, we calculated frequency of virulence to each R-gene or R-gene combination as: $p=x/N$, where x is the number of times the a virulent reaction type was detected and N is the total number of samples tested. We calculated binomial variance of R-gene frequency and compared 95% confidence intervals of the virulence frequencies for the two sets of isolates.

Qualitative resistance of *ex situ* and *in situ* collections

Qualitative (gene-for-gene type) resistance of the barley collections to isolates of *Bgh* was assessed using detached leaf assays, as described in the section on pathotype determination. In this case, however, we grew seedlings from both the 1985 (*ex situ*) and 2008 (*in situ*) collections. In the Petri dishes, we included control leaf segments of a susceptible variety (Rabat 071) and a resistant variety (Taffa) to verify the efficacy of inoculation and spore viability. The inoculated Petri dishes were then incubated and reaction types were assessed, as previously described. An average of ten barley seeds per site were tested against each of the eight *Bgh* isolates (two isolates from each of the *Bgh* sampling sites (Tao.1, Tao.3, Tao.4/Tao.5 and Tao.6) for a total of 768 inoculations. All assays were replicated twice to verify the reaction type. In the case of a discrepancy between replicates, a third test was conducted and the more frequent reaction type

was used. Reaction types from 0 to 2-3 were categorized as resistant (R), while reaction types from categories 3 to 4 were categorized as susceptible (S). The observed frequency of R and S reaction types was calculated for each combination of barley collection, *Bgh* isolate and year. We tested for differences in the distribution of reaction types between years for each collection and isolate using a Chi-squared test.

Qualitative resistance: R-genes present in barley:

To determine the R-genes present in the barley samples we used a set of 50 reference isolates of *Bgh* held in the pathogen genebank at Agricultural Research Institute Kromeriz (Czech Republic). Octal pathotype designation of the isolates was derived from their virulence patterns corresponding to twelve *Ml* resistance genes in coded triplets (Limpert and Muller, 1994) in the order: *a1*, *a3*, *a6*; *a7*, *a9*, *a12*, *a13*, *k1*, *La*, *g*, *at* and *(Bw)* (Appendix III, Table S5).

Detached leaf assays were conducted at Agricultural Research Institute Kromeriz. We tested 42 samples from 1985 and 42 samples from 2008, in the sites Tao.1, Tao.3, Tao.4, Tao.5 and Tao.6. Approximately 50 seeds of each barley accession were sown in two pots (80mm diameter) filled with a gardening peat substrate and placed in a mildew-proof greenhouse under natural daylight. Leaf segments 20mm long were cut from the central part of healthy fully expanded primary leaves. Three leaf segments of each accession were placed in a Petri dish on water agar (0.8% and 40 ppm benzimidazole) for testing with each isolate. Before inoculation, each isolate was purified, verified for the correct virulence phenotype on standard barley lines and increased on leaf segments

of a susceptible line B-3213. For each isolate, a Petri dish with leaf segments was placed at the bottom of a metal inoculation tower and inoculated at an inoculum density of ca. 8 conidia mm^{-2}. The dishes with inoculated leaf segments were incubated at 18±2°C under artificial light (cool-white fluorescent lamps providing 12 h light at 30±5 μmol m^{-2} per s).

Eight days after inoculation, reaction types (RTs) on the upper part of the adaxial side of leaf segments were scored on a nine-point scale, as previously described. Each cultivar was tested in two replications. In the rare cases where leaf segments with more than one distinct RT occurred, the more frequent RT was used for analysis. A set of 50 RTs for each isolate provided the basis for a resistance spectrum (RS) (i.e., a compilation of the R-genes present) of each cultivar. RTs from 0 to 2-3 were categorized as plant resistance (R), indicating the presence of the given R-gene, while reaction types from categories 3 to 4 were categorized as susceptible (S), indicating the absence of that R-gene. The resistance in each cultivar was postulated by comparing the resistance spectrum with previously determined resistance spectra of standard barley lines possessing known resistance genes.

The presence or absence of each R-gene was treated as a binomial variable. Because the samples are all homozygous (due to high levels of natural inbreeding in this species) this can be considered equivalent to a haploid model. We calculated the frequency and binomial variance of each R-gene per site and per year. We tested for differences in R-gene frequency between years (with sites as replicates) using Student's t-test. To compare the distribution of the different resistance spectra in the *in situ* and *ex situ* collections we calculated the frequency

of each resistance spectrum in each collection year and compared them using Student's t-test.

Quantitative resistance: Field trial

Quantitative (polygenic) resistance of the seed collections to *Bgh* was assessed in a field trial that measured powdery mildew infection and disease progression on barley plants. The field trial was conducted at Institut Agronomique et Vétérinaire Hassan II (Rabat, Morocco) from January-March, 2009. We tested geographically paired samples from the collection sites. There was one pair of samples from each of sites Tao.1, Tao.3, Tao.4 and Tao.6, and two pairs of samples from site Tao.5, for a total of 12 samples (six samples from 1985 and six samples from 2008). A randomized complete block design, with a total of five blocks was used. Each block contained one replicate of each of the twelve samples. Samples were planted in 15-seed hill plots (Walsh et al. 1976) with a distance of 30 cm between hills for each experimental unit. Infection with *Bgh* occurred via natural inoculation. All blocks were separated by 3 rows of a susceptible spreader variety (Rabat 071) and the entire plot was surrounded by 3 rows of Rabat 071 to increase natural inoculation and spreading of infection.

The percentage of powdery mildew infection on the first four leaves of plants was estimated visually. Infection was assessed weekly for five plants per hill plot once plants reached the four-leaf stage (GS=14) (James, 1971). A total of five readings were taken. Prior to estimating the percentage of *Bgh* infection in the field, training of field workers for accurate visual estimation of the leaf area covered with powdery mildew was conducted using DISTRAIN, a software

program that evaluates the accuracy and consistency of visual estimation of disease coverage (Tomerlin and Howell, 1988). Training was completed when all readings were at least 90% accurate. In the field, one person conducted all the readings to further minimize variation. Readings were "blind", meaning that the person did not know the identity of the sample being scored, to eliminate potential bias. Area under the disease progress curve (AUDPC) was calculated as:

$$\sum_{i=1}^{n}\left[\left(Y_{i+n_1}+Y_i\right)/2\right]\left[X_{i+1}-X_i\right]$$

where Y_i is mildew severity (per unit) at the ith observation, X_i is the time in days at the ith observation n is the total number of observations and n_1 is the first observation (Shaner and Finney, 1997; Jeger and Viljanen-Rollinson, 2001). AUDPC values were analyzed using a factorial ANOVA with collection year and collection site as factors and with a RCB design. Pairwise comparisons were done using Tukey's HSD test.

Results

Characterization of *Bgh* isolates used in the qualitative and quantitative resistance trials

The *Bgh* isolates used for the qualitative resistance assay (*in vitro* assays) had a high virulence complexity, with nine to eleven virulence genes per isolate. There were seven distinct pathotypes (combinations of virulence genes) among the eight isolates, with pathotype 60042706 occurring in both sites Tao.1 and Tao.6 (Table 2). Most isolates were virulent to the R-genes *a8, a3, a10 + (Du2), ra, k1, nn, at, h* and *(Ab)* (Appendix III, Fig. S2).

The four isolates from the field plot all had distinct pathotypes with virulence to four to ten of the NILs per isolate (Table 2), indicating that field infection is caused by the simultaneous development of a genetically diverse population of *Bgh* spores, rather than by a single pathotype that rapidly multiplied and infected the field. This is further supported by the pathotypes of the 15 *Bgh* isolates from the Rabat region near the field plot, all of which were unique and had virulence to resistance genes in six to eleven of the NILs per isolate. These isolates had similar pathotypes and virulence frequencies to those used in the detached leaf assay (Appendix III, Fig.S2). Overall, these isolates can be considered to have similar pathotypes, and the differences observed between the isolates should therefore not be a confounding variable when comparing the results of the field trials with the results of the qualitative resistance (detached leaf) trials. The isolates characterized had different pathotypes and frequencies of R-genes than isolates described for the region in 1992 (Yahyaoui et al., 1997). This indicates that there has been evolution of isolate pathotypes in the region over the time period discussed in this paper.

Qualitative resistance of barley to *Bgh* isolates

The qualitative reaction types **(**resistant and susceptible) to eight different *Bgh* isolates were compared for five separate geographically paired samples of *ex situ* and *in situ* conserved samples (Fig. 2). Of the forty combinations of isolate and seed collection, there were five collection by isolate combinations that had a significant difference in resistance between *in situ* and *ex situ* collections. These were: isolate Pm.34 and site Tao.3 (*ex situ* more resistant than *in situ*), isolate Pm.

68 and site Tao.3 (*ex situ* more resistant than *in situ*), isolate Pm.200 and site Tao.4 (*ex situ* more resistant than *in situ*), isolate Pm.223 and site Tao.5 (*ex situ* more resistant than *in situ*) and isolate Pm.234 and site Tao.1 (*in situ* more resistant than *ex situ*). In 13 of the 40 isolate by seed collection combinations all the seedlings from at least one of the collections were susceptible. Of these, in six cases the *ex situ* collection was entirely susceptible, in another six cases the *in situ* collection was entirely susceptible and in one case both *ex situ* and *in situ* collections were entirely susceptible.

Qualitative resistance: R-genes present in barley:

The barley collections from 1985 and 2008 contained a total of 29 R-genes. Of these, 16 were present in 1985 and 21 were present in 2008. Each individual accession tested had between 0 and 3 R-genes. The mean number of R-genes in the resistance spectra of the accessions remained similar between years, averaging 1.4 (+/-0.6) in 1985 and 1.6 (+/-0.6) in 2008. The frequencies of the resistance genes that were present in both years (and could therefore be compared using a t-test; resistance genes *a8, at, (Ch), u3, u11, ra* and *h*) did not change significantly between years. Resistance gene *a8* was the most common (frequency = 0.6 in both 1985 and 2008) and was present in both years and in all sites (Fig. 3). The resistance gene "*g"* was detected for the first time in 2008, where it occurred at intermediate frequencies (0.22 and 0.33) in sites Tao.1 and Tao.6, respectively. A total of sixteen distinct, but unidentified, R-genes (*u1-u16*) were present in the germplasm at low frequencies (0.10 or less).

The *a8* gene occurred in the most common resistance spectra (i.e., combinations of resistance genes in a single sample), either alone or in combination with other R-genes, including *at*, *u3* and *u11*, (Fig. 4). There were eight resistance spectra that were present in both years: *a8; (Ch); a8+u11; a8 + at; a8 +u3; u16; at* and "none", the later indicating accessions with no detected R-genes. The frequencies of these resistance spectra were not significantly different between years in t-tests. In contrast to the common R-genes and their resistance spectra, the identity of rare resistance genes and rare resistance spectra showed a complete turnover between 1985 and 2008. This is not unexpected by chance alone, given the low frequencies of these genes.

Quantitative resistance of barley to *Bgh*

Area under the disease progress curve (AUDPC) was used as a measure of quantitative disease resistance in field trials. AUDPC values ranged from 37.6 to 719.0, with a median value of 287.1 (higher AUDPC indicates lower quantitative resistance). ANOVA analysis revealed that both collection year ($F=18.3923$, $df=1$, $P=0.0001$) and sampling site ($F=6.2046$, $Df=5$, $P=0.0002$) were significant factors with regards to AUDPC, as was the interaction term ($F=23.7394$, $Df=5$, $P<0.00001$) (Table 3). In two of the 2008 (*in situ*) collections (sites Tao.3 and Tao.6), quantitative resistance was significantly greater than in the respective paired 1985 (*ex situ*) samples, whereas for one of the collection pairs (site Tao.5.1), the *ex situ* collection had higher resistance (Fig. 5)

Discussion

We identified differences in *Bgh*-resistance between barley collections conserved *in situ* and *ex situ.* These differences only rarely involved the gene-for-gene system (qualitative resistance) of host-pathogen interactions (Jones and Dangl, 2006) with only five out of forty significant differences in resistance between collections. This was reflected in the lack of differences in the frequencies of major R-genes in the host germplasm, although minor genes changed in identity and the *Mlg* R-gene appeared in the *in situ* germplasm. We also found changes in quantitative resistance between some of the collections in the field trials, with the highest quantitative resistance being in the *in situ* collections.

These findings provide some support for our original expectations, namely that qualitative resistance can either decrease *ex situ* (due to lag load exacerbated by storage of germplasm away from the natural habitat) or increase *ex situ* (due to rare R-genes being preserved *ex situ* but lost *in situ*), while quantitative resistance should increase in the *in situ* collections. We saw evidence of both of these expected trends in both types of resistance. It is of note that *ex situ* collections appeared to conserve a greater number of adaptive genes for qualitative resistance compared to *in situ* collections, possibly due to the preservation of rare R-genes to which the pathogen has lost virulence. This is contrary to the general assumption that only *in situ* collections maintain adaptive resistance and confirms our hypothesis that removal from the environment may, in some cases, provide a mechanism to conserve useful and adaptive resistance. In other cases, *in situ* conservation resulted in higher resistance, consistent with the established

paradigm. However, the small number of differences between *in situ* and *ex situ* germplasm for qualitative resistance was unexpected. With the possibility of type I error expected to account for approximately two of these significant results, the number of significant differences is effectively very small.

One possible explanation for the small number of differences in qualitative resistance between *in situ* and *ex situ* collections is that the evolutionary dynamics of the host-pathogen interaction studied here do not conform as closely as expected to the classic gene-for-gene model. In order for gene-for-gene interactions to bring about an "evolutionary arms race", there must be sufficient selective pressure and sufficient genetic variation in the co-evolving system (Bergelson et al. 2001). Selective pressure is provided by the presence of disease (causing yield losses of up to 30% in North Africa) (Caddel and Wilcoxson, 1975; Amri et al., 2002) and the frequent appearance of novel virulence within the pathogen population. Resistance variation is provided by a large pool of R-genes in the host populations, or by the capacity to acquire these new genes via gene flow or *de novo* mutation. The pathogen in our study conformed to these assumptions, and exhibited significant turnover in pathotypes within the time period studied (Yahyaoui et al., 1997; Jensen et al., 2013). However, the host apparently did not conform well to these expectations. The host population had a high number of resistance genes, but lacked several major genes identified in other populations of traditional Moroccan barley such as *a12* and *a6 + a14* (Czembor and Czembor, 2000). Furthermore, most of these genes were present at low frequencies. This relatively low diversity and frequency of R-genes within the host population may have reduced the strength of frequency-dependent selection

and cycling of R-genes and virulence genes over the twenty-three year period of this study.

Another possible reason why we did not frequently detect lower resistance in the *ex situ* maintained materials could be our restriction of comparisons to collections pairs where we were confident that the *in situ* collection had evolved in the same site as the *ex situ* collection (based on Fst values). As discussed earlier, this was done to avoid comparing *ex situ* maintained materials with newly introduced on-farm germplasm that may have replaced those made in the 1985 collection and was not subject to selection pressures *in situ*. It is possible that divergence in microsatellite allele frequencies could have come about as the result of hitchhiking effects associated with selection for pathogen resistance that occurred in the 23 years separating the two collections. We believe this to be unlikely, for two reasons. First, linkage is weak between known R-genes and the microsatellites used in this study (Varshney et al., 2007; Aghnoum et al., 2010) (Appendix III, Table S2). And second, to drive Fst to the significantly different levels seen in the excluded materials (Fst = 0.2-0.3), hitchhiking and selection during the short intervening period when the two collections were made would have had to be intense for many R-genes.

The most abundant R-genes present in *ex situ* and *in situ* collections (*a8, at, (Ch), u, u3*) remained unchanged in frequency in both collections. The pathogens had high frequencies of virulence to R-genes *a8* and *at* (data for (*Ch*), *u* and *u3* are not available), making these R-genes ineffective for preventing infection. However, there were several potentially adaptive changes in host resistance. The R-gene "*g*" increased in frequency from 0% in 1985 to 10% in

2008. Because 82% of the pathotypes in this region are avirulent to "*g"* (Jensen et al., 2013), this would be a relatively effective source of resistance and is a potentially adaptive change in the host germplasm. The R-gene *a6* was only identified in the *in situ* collections, and only one of the isolates was virulent to it. Despite these potentially adaptive changes, there does not appear to be a tight co-evolution between host R-genes and pathogen avirulence genes in the *in situ* populations.

Although the major R-genes remain unchanged, the rare R-genes in the germplasm, (mostly the unidentified "*u*" genes) showed complete turnover in identity, with 13 new R-genes detected in 2008 compared to 1985. There are two possible explanations for this result. First, gene flow from other collections, possibly European barley varieties, introduced new R-genes into the germplasm, while rare R-genes that are no longer present *in situ* were retained in the *ex situ* collection. Second, because these genes are rare, our sample was not large enough to detect them in both years. Further tests are required to determine which is the correct explanation.

With regards to quantitative resistance, our study identified significant differences between several of the geographically paired *in situ* and *ex situ* collections that we tested. Two of the *in situ* conserved collections showed higher quantitative resistance to *Bgh* than the associated *ex situ* collections. Conversely, one *ex situ* collection had higher quantitative resistance than the *in situ* collection. One of the *in situ* collections (from the Tao.6 site) had much lower disease levels than any of the other *in situ* or *ex situ* collections, indicating a potentially interesting and novel source of quantitative resistance derived via *in situ*

conservation. These results are paralleled by reports in the breeding literature that bulked hybrid populations of crop plants planted year after year in the same site show an increase in quantitative resistance. This pattern is seen for barley and powdery mildew (Ibrahim and Barrett, 1991), wheat and powdery mildew (Le Boulc'h et al., 1994; Paillard et al., 2000a; Paillard et al., 2000b) as well as for host-pathogen systems in other crops (Horneburg and Becker, 2008). Although we have observed changes in quantitative resistance in two *in situ* collections, it is difficult to ascertain whether this is due to selection or is a by-product of neutral processes such as genetic drift or gene flow. However, in a wild host-pathogen system (flax and flax rust), quantitative resistance was subject to adaptive co-evolution in a similar manner to qualitative resistance (Antonovics et al., 2011), despite the difference in genetic mechanisms. The evolutionary maintenance of quantitative resistance is an agronomically important trait for crop genetic resource management because it has the potential to confer durable resistance. Quantitative resistance indicates the action of genes that work after a successful infection by the pathogen to limit damage to the plant and induce resistance to further infection. This type of resistance is considered to be equally effective against numerous pathogen isolates and is less vulnerable to the evolution of novel virulence genes in the pathogen. For these reasons, plant breeders place a high value on quantitative resistance (compared to qualitative resistance) (Murphy et al., 2005).

It is of note that the majority of the barley samples tested were moderately or highly susceptible to *Bgh*. Furthermore, although 29 R-genes were detected, only four of these had frequencies above 10% and only one (*a8*) had a frequency

above 50%. Farmers report that they use the *Beldi* variety because it is adapted to local conditions, including poor soil, rain-fed cultivation and periodic droughts. Experimental data have demonstrated that traditional Moroccan varieties consistently outperform registered Moroccan varieties in terms of drought stress resistance (Oukarroum et al., 2007). The *Beldi* variety is also robust to the practice of green stage grazing (Anderson, 1985; Yau and Mekni, 1987) and yields high-quality straw used for livestock feed that represents a significant proportion of the crop value (Annicchiarico and Pecetti, 2003; Grando et al., 2005). The finding that *Beldi* is susceptible to many *Bgh* isolates suggests that farmers could benefit from participatory breeding efforts to improve the qualitative and quantitative resistance of this germplasm, while preserving these other desirable and culturally adapted traits (Bellon, 1996; Brush and Meng, 1998; Phillips and Wolfe, 2005).

Overall, this study highlights different adaptive outcomes of conserving seeds *ex situ* compared to conserving seeds *in situ*. From the genetic resource management perspective, this demonstrates the importance and complementarity of the two types of conservation. Conservation policies should therefore include both *in situ* and *ex situ* conservation strategies. Furthermore, the differences we have seen between qualitative and quantitative resistance of collections underscore the importance of assessing both types of resistance when evaluating the efficacy of different conservation methods for maintaining pathogen resistance in crop germplasm. The maintenance of multilocus (quantitative) resistance to pathogen populations is likely best achieved by recurrent mass selection of a large and heterogeneous population, and is thus more likely to occur on-farm (*in situ*).

This process can potentially be enhanced using participatory and on-farm breeding techniques (Dawson et al., 2008). However, in some cases, *ex situ* collections may preserve some specific alleles that have been lost from *in situ* populations.

Future work on this topic should include expanding the components of this study to plant-pathogen systems in different traditional farming systems in order to test the generality of the conclusions regarding *in situ* and *ex situ* conservation. In particular, it would be interesting to examine crops with different mating systems, such as outcrossing and clonally propagated crops. Further study of changes in quantitative resistance of *in situ* and *ex situ* materials, with a larger number of samples and covering a broader geographic region, could provide important insights.

The emergence and dissemination of novel pathogen virulence and host resistance is a stochastic and unpredictable process that can have devastating impacts on crop yields. This was recently exemplified by emergence of a novel race of wheat rust (UG99) in Uganda (Singh et al., 2007) with virulence the majority of the resistance genes deployed in cultivated wheat (Jin et al., 2009). This race has spread to the Middle East and has the potential to affect the major wheat-growing areas in Asia and potentially in North America (Nazari et al., 2009). In the case of this pathogen, the search for sources of resistance has encompassed both modern and traditional crop varieties (Sidiqi et al., 2009) and both qualitative and quantitative sources of resistance (Singh et al., 2011). Our study demonstrates, from an evolutionary perspective, the importance of maintaining both *in situ* and *ex situ* collections of traditional crop varieties for use

in the defense against novel pathogen races. *In situ* collections had higher resistance in some cases, and showed some potential changes in R-gene frequency and identity. Conversely, although some of the *ex situ* collections suffered from lag load, as could be expected under the Red Queen scenario, others had high resistance and, furthermore, they may retain rare resistance alleles that can be useful in future breeding applications.

Tables

Table 1. Differential varieties of barley and their associated resistance genes.

Differential	Resistance gene(s)[a]	Triplet[b]
Pallas	*Mla8*	1
P02	*Mla3*	1
P03	*Mla6, Mla14*	1
P04A	*Mla7, Mlk1, MlaNo3*	2
P04B	*Mla7, MlaNo3*	2
P06	*Mla7, MlaMu2*	2
P07	*Mla9, Mlk1*	3
P08A	*Mla9, Mlk1*	3
P08B	*Mla9*	3
P09	*Mla10, MlaDu2*	4
P10	*Mla12, MlaEm2*	4
P12	*Mla22*	4
P13	*Mla23*	5
P14	*Mlra*	5
P15	*Ml(Ru2)*	5
P17	*Mlk1*	6
P18	*Mlnn*	6
P20	*Mlat*	6
P21	*Mlg, Ml(CP)*	7
P22	*mlo5*	7
P23	*MlLa*	7
P24	*Mlh*	8
Lotta	*Ml(Ab)*	8
Triumph	*Mla7, Ml(Ab), MlaTr3*	8

[a]Gene names in parentheses (e.g., *Ml(Ru2)*) refer to R-genes have not yet been genetically mapped.

[b]Triplet assignment used for coding the pathotype.

Table 2. Characteristics of the *Bgh* isolates used in the *in vitro* assays and in the field trial.

Isolate	**Site**	**Pathotype**[a]	**Complexity**[b]	**Experiment**[c]
Pm.200	Tao.1	60013704	9	*in vitro* assays
Pm.202	Tao.1	60042706	9	*in vitro* assays
Pm.209	Tao.3	40012746	9	*in vitro* assays
Pm.034	Tao.3	70044614	9	*in vitro* assays
Pm.223	Tao.4/Tao.5	60042716	10	*in vitro* assays
Pm.234	Tao.4/Tao.5	60043706	10	*in vitro* assays
Pm.241	Tao.6	60042706	9	*in vitro* assays
Pm.068	Tao.6	60044706	9	*in vitro* assays
Pm.114	Rabat	20043704	8	Field trial region
Pm.116	Rabat	40040706	7	Field trial region
Pm.092	Rabat	40042004	4	Field trial site
Pm.113	Rabat	40042516	8	Field trial region
Pm.118	Rabat	40042704	7	Field trial region
Pm.090	Rabat	40042744	8	Field trial site
Pm.089	Rabat	40043716	10	Field trial site
Pm.106	Rabat	40046506	8	Field trial region
Pm.117	Rabat	40047506	9	Field trial region
Pm.115	Rabat	60010106	6	Field trial region
Pm.124	Rabat	60010704	7	Field trial region
Pm.123	Rabat	60012544	8	Field trial region
Pm.088	Rabat	60042504	7	Field trial site
Pm.120	Rabat	60042506	8	Field trial region
Pm.119	Rabat	60042507	9	Field trial region
Pm.107	Rabat	60042704	8	Field trial region
Pm.111	Rabat	60042706	9	Field trial region
Pm.112	Rabat	60052704	9	Field trial region
Pm.121	Rabat	70013546	11	Field trial region

Table 2 (cont.)

[a] Each pathotype represents a distinct combination of virulence genes.

[b] The total number of virulent reaction types per isolate

[c] "Field trial site" indicates that the isolate was collected from the actual field trial, "Field trial region" indicates that the isolate was collected from the same region as the field trial.

Table 3. Two-way analysis of variance for AUDPC testing the effects of year collected (i.e., 1985 vs. 2008) and collection site.

	Df	Sum Sq	Mean Sq	F-value	Pr
Year	1	133058	133058	18.3923	P<0.0001
Site	5	224433	44887	6.2046	P<0.0001
Year * Site	5	858703	171741	23.7394	P<0.0001
Residuals	44	318314	7234		

Figures

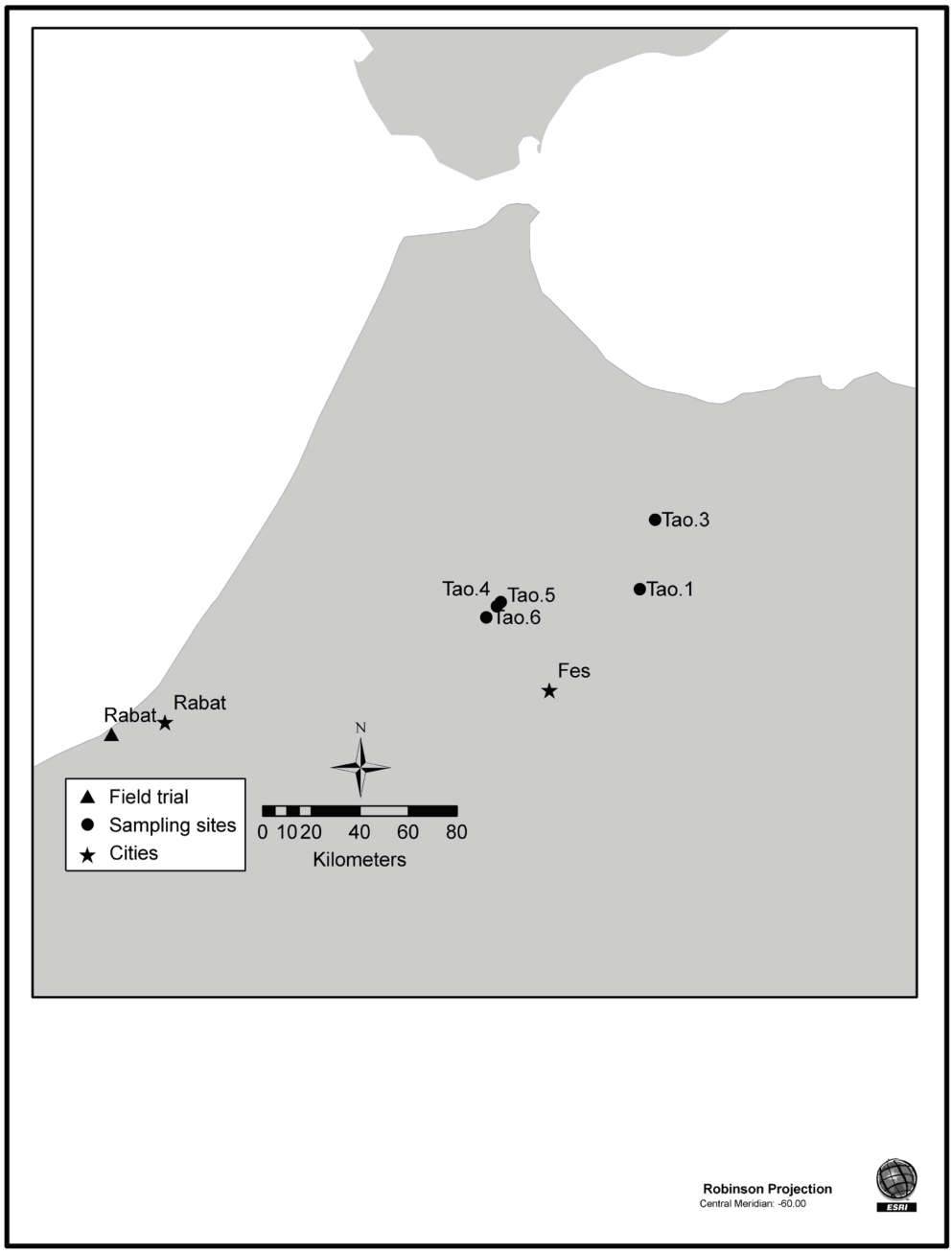
Tao.3
Tao.4
Tao.5
Tao.6
Tao.1
Fes
Rabat
Rabat
N
Field trial
Sampling sites
Cities
0 10 20 40 60 80
Kilometers
Robinson Projection
Central Meridian: -60.00
ESRI

Figure 1. Sampling sites in Morocco for barley seeds and *Bgh* isolates as well as for conducting the field trial. Circle symbols (•) denote sites sampled for barley seeds in 1985 (by ICARDA) and re-sampled in 2008 (site codes are Tao.1, Tao.3, Tao.4, Tao.5 and Tao.6). The triangle symbol (▲) shows the site of the field trial in Rabat. Sampling sites for *Bgh* isolates correspond to the barley sampling sites Tao.1, Tao.3, Tao.4, Tao.6 as well as Rabat. Site agro-climatic zones are defined according to the system of the United Nations Food and Agriculture Organisation where the first two letters denote the moisture regime (SH=subhumid, SA=semi-arid), the second letter denotes the winter type (C=cool, M=mild) and the third letter denotes the summer type (W=warm) (FAO 2006). Site Tao.1 is zone SH-M-W, site Tao.3 is zone SH-C-W, sites Tao.4, Tao.5 and Tao.6 are SA-M-W and Rabat is SH-W-W. The lowest elevation site (Rabat) is located at 23m and the highest elevation site (Tao.3) is located at 796m.

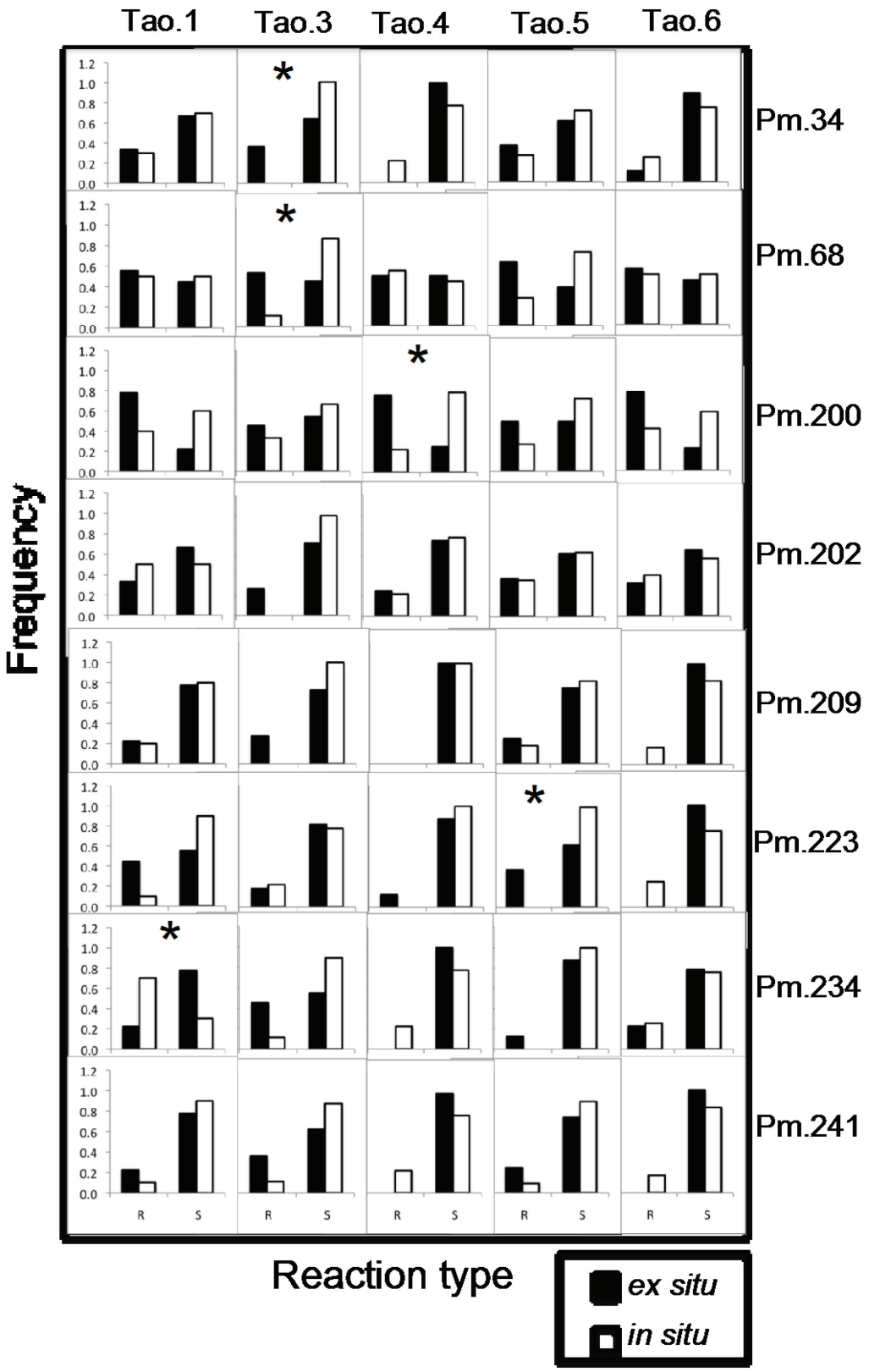
Tao.1
Tao.3
Tao.4
Tao.5
Tao.6
Pm.34
Pm.68
Pm.200
Pm.202
Pm.209
Pm.223
Pm.234
Pm.241
Frequency
Reaction type
R
S
ex situ
in situ

Figure 2. Qualitative resistance of geographically paired barley collections from 1985 (*ex situ*) and 2008 (*in situ*) to eight different *Bgh* isolates. Black bars denote ex situ collections while white bars denote in situ collections. Asterisk indicates significant difference ($p<0.05$) between the *ex situ* and *in situ* collections. There are two possible reaction types: resistant (R) or susceptible (S).

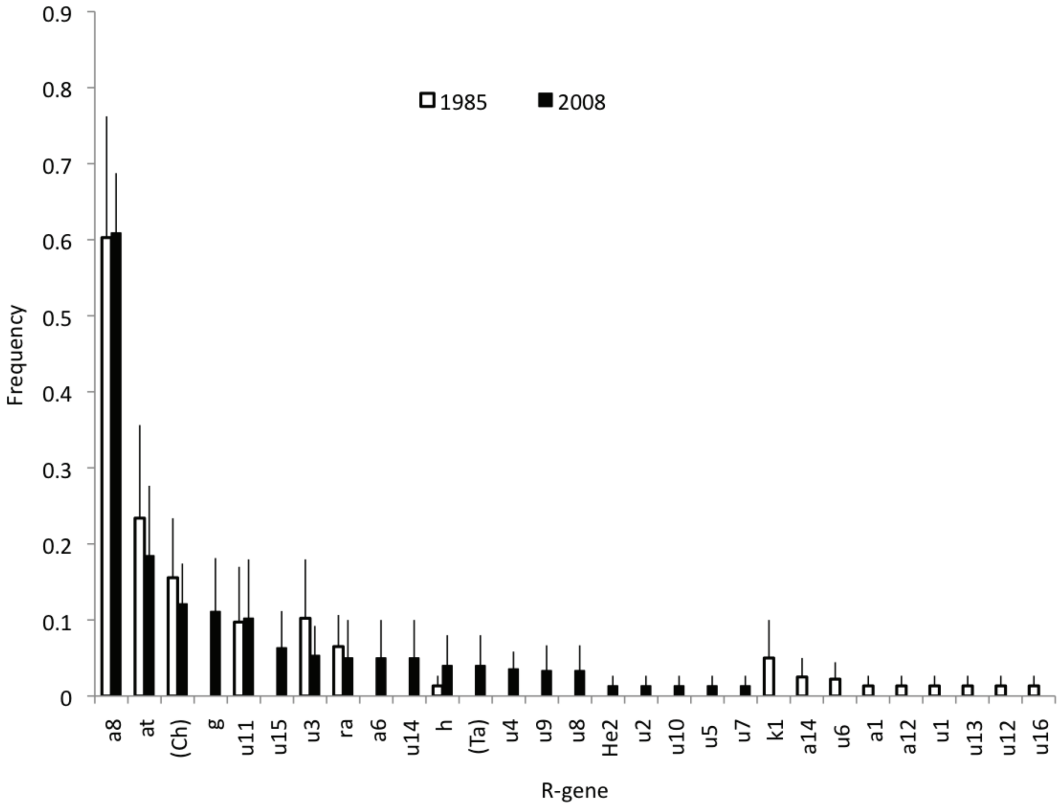

Figure 3. Resistance-genes present in barley samples from 1985 (*ex situ*) and 2008 (*in situ*). Bars represent the mean frequency of the five sites (+ SE). There were no significant differences in frequencies between years.

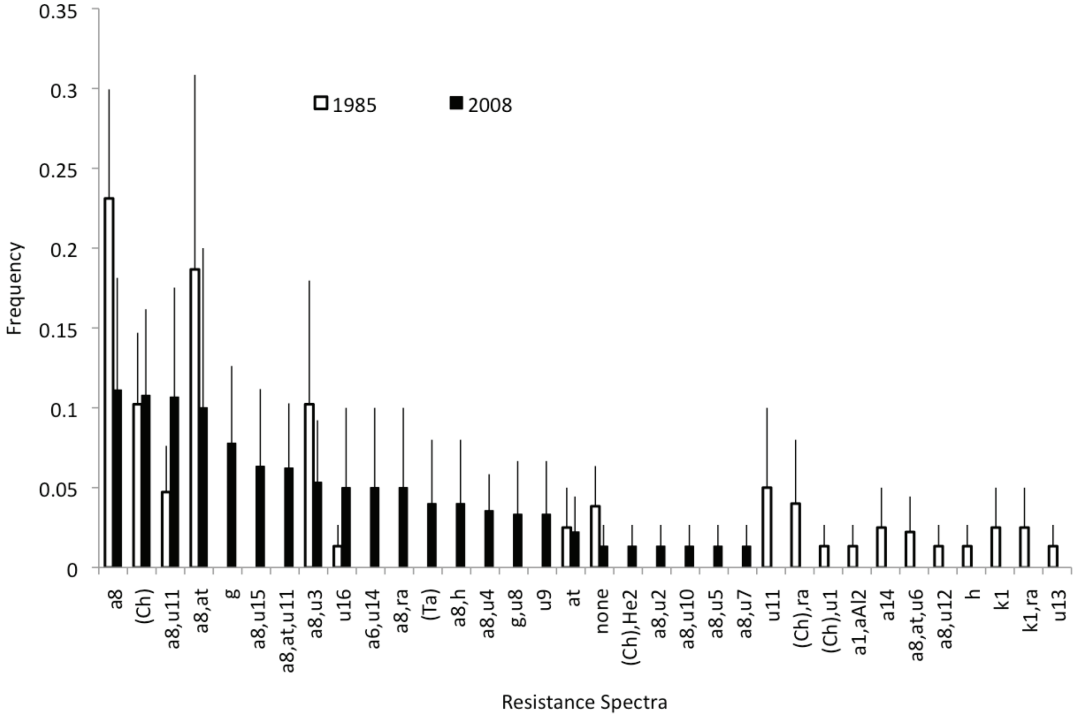

Figure 4. Resistance gene spectra (combinations of resistance genes) present in barley samples from 1985 and 2008. Bars represent the mean frequency of the five sites (+ SE). There were no significant differences in frequencies between years. Some samples contain only one R-gene, resulting in a resistance spectrum with only one R-gene. R-genes with prefix "u" represent genes that are unidentified and are known only by the reaction spectrum of reference barley varieties to reference *Bgh* isolates. R-gene names in parentheses (e.g., *Ml(Ru2)*) refer to R-genes that have been identified in specific genetic backgrounds, but have not yet been genetically mapped.

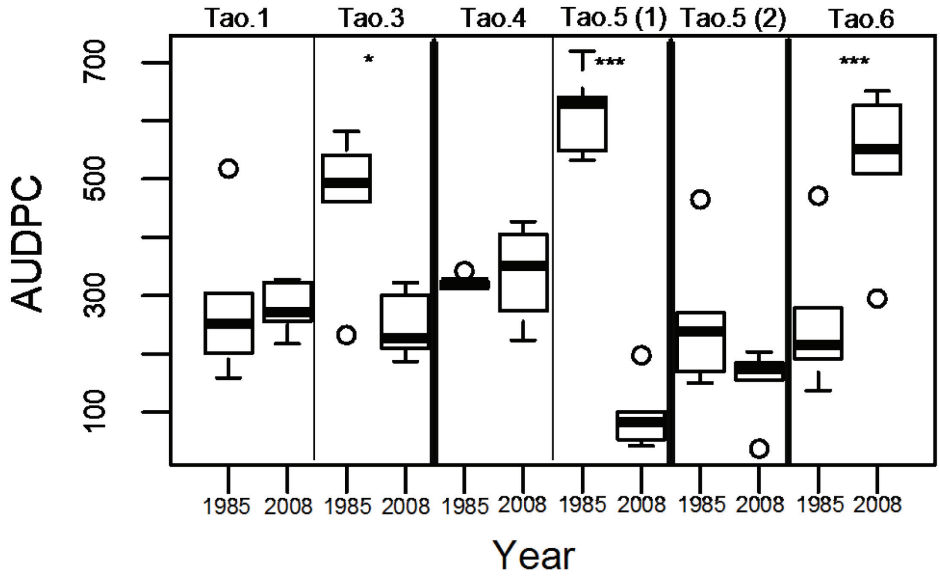

Figure 5. Quantitative resistance of paired barley collections from 1985 (*ex situ*) and 2008 (*in situ*) to *Bgh.* Resistance is measured as area under the disease progress curve (AUDPC) following natural inoculation with *Bgh* in field plots in the Rabat region. Each panel denotes collections from one site compared between years. Asterisks denote significant differences between *ex situ* and *in situ* samples from a given site (*p<0.05, ***p<0.001).

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GENERAL CONCLUSIONS

The interaction between humans and domesticated crops is a dynamic and ongoing process, rather than a single, rapid transition from a wild to a domesticated species. In chapter 1, I conclude that many established theories with regards to crop domestication are the result of an overemphasis on "model" crops in the study of domestication and that commonly accepted domestication syndromes such as loss of shattering and shifts in breeding system are less common within a broader dataset.

In chapter 2, I initiated a detailed study of one particular crop, barley in northern Morocco. Although many authors have described genetic diversity and structure of traditional crops and others have described farmer perceptions of genetic diversity and seed movement through seed exchange networks (e.g., Soleri and Cleveland, Pautasso et al., 2012), it preferable to assess both genetic structure and seed exchange patterns concurrently. Here I provide one of the few studies to take this approach (e.g., Delêtre et al., 2011) and I provide the first comprehensive description of seed exchange networks for barley in this region, coupled with measurements of genetic diversity, structure and gene flow among populations of a complex of local barley varieties. I demonstrate that farmers in this region value traditional barley varieties because of a suite of traits including productivity, local adaptation, drought resistance, and quality of animal fodder that are consistent with their priorities for this crop. I also determine that the

majority of crop seed is conserved on-farm with some inputs from local markets and seed companies.

In chapter 3, I extended my study of traditional barley varieties to a larger geographic region in Northern Morocco and included temporal changes over a 23-year period. I also assessed the diversity and structure of seed available in local markets and the extent to which gene flow from markets was detectable in farm populations of barley. Major conclusions from this work are that spatial genetic structure has decreased with time in traditional varieties of barley. However, seeds conserved on-farm remain distinct from market seeds, indicating that on-farm seed conservation is effective.

In chapter 4, I investigate virulence diversity and distribution in the pathogen *Blumeria graminis* f.sp. *hordei* (*Bgh*). Conclusions from this work include that the population of *Bgh* is highly diverse and highly differentiated from contemporary populations in Europe. There is some evidence that virulence in the *Bgh* population is correlated with resistance genes present in the barley population.

In chapter 5, I test the prediction that barley populations maintained *in situ* will have improved resistance to populations of a co-occurring pathogen compared to populations maintained *ex situ*. Conclusions from this study indicate that this is not always the case, with accessions from both *in situ* and *ex situ* collections showing improved qualitative and quantitative resistance.

The research reported in this thesis has implications for the conservation of traditional crop varieties in centers of diversity. In particular, the results from this work can help to determine the appropriate scale for sampling barley diversity

in the study region. On a broader level, this research has used innovative approaches to study crop diversity on a spatial and temporal scale that could be applied to other crops. Most importantly, it has been demonstrated that conservation *in situ* appears to increase quantitative resistance of barley populations, whereas conservation *ex situ* appears to favour qualitative resistance. This later result is contrary to assumptions common in the literature for crop genetic resources.

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APPENDIX I

Supporting information for chapter one is available in the online version of the article published in *The New Phytologist* **196**: 29–48 (DOI: 10.1111/j.1469-8137.2012.04253.x). More recent versions of tables S2, S4 and S5 that are periodically updated as new information becomes available may be found at: www.cropdomestication.com

Table S1. Online databases accessed to supplement crop information from the literature.

Table S2. Literature cited for each of the 203 reviewed food crops.

Table S3. Definitions, rationales and abbreviations used for coding categories and subcategories.

Table S4. Boolean matrix of domestication data for 203 food crops.

Table S5. Annotated matrix to supplement the Boolean matrix of domestication data (Table S4) with more specific dates for plant exploitation and domestication, life cycle, ploidy, and geographic center of domestication.

Table S6. Searchable and sortable heat map of Logic Forest importance values. Positive values indicate positive correlations (color-coded blue and grey),

negative values indicate negative correlations (color-coded orange and pink). Boxes with an “x” indicate correlations that are not of interest. Definitions of abbreviations and descriptions of category delimitations are available in Table S3.

APPENDIX II

Table S1: Infection type classification for powdery mildew on cereals (from Torp *et al.* 1978).

Infection type	Mycelium growth	Sporulation	Development of chlorosis/necrosis
0	None	None	No
0-1	None	None	Yes
1	Weak	None	Yes
1-2	Weak	Weak	Yes
2	Moderate	Weak	Yes
2-3	Moderate	Moderate	Yes
3	Strong	Moderate	Yes
3-4	Strong	Strong	Yes
4	Strong	Strong	No

Table S2: Pathotypes and virulence complexities of the 72 isolates of *Blumeria graminis* f.sp. *hordei*.

Isolate ID	Pathotype	Freq (%)	Complexity[a]	Site(s)
Pm.111, Pm.202, Pm.235, Pm.241	60042706	5.56	9	Rabat, Tao.1, Tao.4, Tao.6
Pm.119, Pm.120, Pm.210	60042506	4.17	8	Rabat, Rabat, Tao.3
Pm.107, Pm.222, Pm.236	60042704	4.17	8	Rabat, Tao.3, Tao.4
Pm.232, Pm.237, Pm.239	60040704	4.17	7	Tao.4, Tao.4, Tao.6
Pm.225, Pm.226	60041716	2.78	10	Tao.4, Tao.4
Pm.234, Pm.246	60043706	2.78	10	Tao.4, Tao.6
Pm.231	70047706	1.39	12	Tao.4
Pm.121	70013546	1.39	11	Rabat
Pm.214	60043746	1.39	11	Tao.3
Pm.216	70046706	1.39	11	Tao.3
Pm.224	40046756	1.39	11	Tao.4
Pm.030	40012756	1.39	10	Tao.3
Pm.089	40043716	1.39	10	Rabat
Pm.211	60053704	1.39	10	Tao.3

Isolate ID	Pathotype	Freq (%)	Complexity[a]	Site(s)
Pm.215	70043306	1.39	10	Tao.3
Pm.221	60042547	1.39	10	Tao.3
Pm.223	60042716	1.39	10	Tao.4
Pm.034	70044614	1.39	9	Tao.3
Pm.068	60044706	1.39	9	Tao.6
Pm.112	60052704	1.39	9	Rabat
Pm.114	60043704	1.39	9	Rabat
Pm.117	40047506	1.39	9	Rabat
Pm.200	60013704	1.39	9	Tao.1
Pm.209	40012746	1.39	9	Tao.3
Pm.218	40042716	1.39	9	Tao.3
Pm.219	60013506	1.39	9	Tao.3
Pm.233	60042744	1.39	9	Tao.4
Pm.066	40013704	1.39	8	Tao.6
Pm.090	40042744	1.39	8	Rabat
Pm.106	40046506	1.39	8	Rabat
Pm.113	40042516	1.39	8	Rabat
Pm.123	60012544	1.39	8	Rabat
Pm.201	60043304	1.39	8	Tao.1
Pm.203	40042706	1.39	8	Tao.1
Pm.205	60052504	1.39	8	Tao.3

Isolate ID	Pathotype	Freq (%)	Complexity[a]	Site(s)
Pm.206	40012516	1.39	8	Tao.3
Pm.212	60040706	1.39	8	Tao.3
Pm.217	60042704	1.39	8	Tao.3
Pm.220	60052700	1.39	8	Tao.3
Pm.227	50012704	1.39	8	Tao.4
Pm.228	60042514	1.39	8	Tao.4
Pm.244	40052704	1.39	8	Tao.6
Pm.245	60003704	1.39	8	Tao.6
Pm.035	50042504	1.39	7	Tao.3
Pm.088	60042504	1.39	7	Rabat
Pm.116	40040706	1.39	7	Rabat
Pm.118	40042704	1.39	7	Rabat
Pm.124	60010704	1.39	7	Rabat
Pm.213	60040344	1.39	7	Tao.3
Pm.229	60000546	1.39	7	Tao.4
Pm.240	60042502	1.39	7	Tao.6
Pm.062	40010614	1.39	6	Tao.6
Pm.065	40050540	1.39	6	Tao.6
Pm.079	40040704	1.39	6	Tao.6
Pm.115	60010106	1.39	6	Rabat
Pm.242	40012304	1.39	6	Tao.6

Isolate ID	Pathotype	Freq (%)	Complexity[a]	Site(s)
Pm.243	60040604	1.39	6	Tao.6
Pm.076	40040304	1.39	5	Tao.6
Pm.238	40002406	1.39	5	Tao.6
Pm.092	40042004	1.39	4	Rabat
Pm.230	40000204	1.39	3	Tao.4

[a]The number of differentials to which the isolate was virulent.

APPENDIX III

Supplementary tables and figures providing information in support of the data presented in the study described in chapter 5.

Table S1: Comparison of barley collections from 1985 and 2008 using qualitative and quantitative morphological traits.

Trait	1985	2008
Row number	6	6
Spike density	Lax	Lax
Lemma awn[1]	3	3
Lemma awn barbs[2]	7	7
Glume/Glume awn length[3]	2	2
Length of rachilla hair	Long	Long
Aleurone layer	White	White

[1] 1=awnless; 2=awnletted; 3=awned; 4=sessile hoods; 5=elevated hoods

[2] 3=smooth (few bars at tip); 5=intermediate (small barbs on upper half); 7=rough (barbs along entire length)

[3] 1=glume + awn shorter than kernel; 2=glume + awn as long as kernel; 3=glume + awn longer than kernel;
4=glume + awn nearly twice as long as kernel; 5=lemma-like (very thick)

Table S2: Names, chromosome, linkage to R-genes, primers sequences, PCR programs and product size range for the microsatellite markers used to characterize the *H. vulgare* samples.

SSR	Chromosome	Closest known R-gene and distance (cM)	Forward primer (with M13 (-43) tail)[1]	Reverse primer	PCR program[2]	Size
Bmag0345	1H	Mlk (19cM)	5'-AGG GTT TTC CCA GTC ACG ACG TTA TGA GGA ATA ACT CAA CCA AA-3'	5'-AAT ATA TTT TCG ATG TCG AGC-3'	M13:F	175
Bmag0125	2H	M1La (66 cM)	5'-AGG GTT TTC CCA GTC ACG ACG TTA ATT AGC GAG AAC AAA ATC AC-3'	5'-AGA TAA CGA TGC ACC ACC-3'	M13:E	180
EBmac0541	3H	No R-genes on chromosome 3H	5'-AGG GTT TTC CCA GTC ACG ACG TTA CGG ATC TAC TTT AGC TAG CA-3'	5'-AAA CAA CCC CAC ACA ATC-3'	M13:F	140
Bmag0013	3H	No R-genes on chromosome 3H	5'-AGG GTT TTC CCA GTC ACG ACG TTT CAA AAG CCG GTC TAA TGC T-3'	5'-GTG CAA AGA AAA TGC ACA GAT AG-3'	M13:E	189
Bmac0316	6H	No R-genes on chromosome 6H	5'-AGG GTT TTC CCA GTC ACG ACG TTA TGG TAG AGG TCC CAA CTG-3'	5'-ATC ACT GCT GTG CCT AGC-3'	M13:E	210
Bmag0321	7H	Mlf (24 cM)	5'-AGG GTT TTC CCA GTC ACG ACG TTA TTA TCT CCT GCA ACA ACC TA-3'	5'-CTC CGG AAC TAC GAC AAG-3'	M13:F	250

[1] M13 (-43) sequence: 5'-AGG GTT TTC CCA GTC ACG ACG TT-3'
[2]See program details in table A3.

Table S3: Details of the PCR programs used for the microsatellite markers.

Program name	Cycle number	Times and conditions
M13-E:	1 cycle:	5 min @ 95C
	10 cycles	1 min @ 94C, 1 min @ 55 C, 1min @ 72C
	27 cycles	30s @ 94C, 1 min @ 55C, 30s @ 72C
	1 cycle:	10 min @ 72C
M13-F:	1 cycle:	5 min @ 95C
	10 cycles	30s @ 94C, 1 min @ 58 C, 30s @ 72C
	27 cycles	30s @ 94C, 1 min @ 55C, 30s @ 72C
	1 cycle:	10 min @ 72C

Table S4: Comparison of the seed samples collected in 2008 to the original seed samples from 1985, using microsatellite data and AMOVA analysis. Note that all comparisons show non-significant Fst and therefore minimal genetic differentiation between sample pairs.

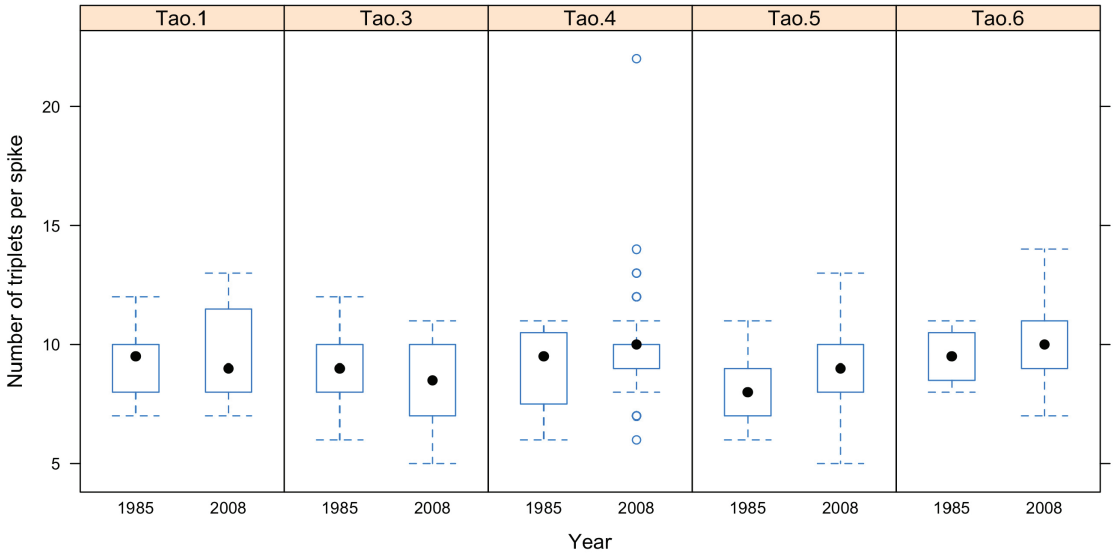
					Inoculations		
Site	Sample	Fst	p	Sig.	Field isolates	Endemic isolates	Reference isolates
Tao.1	170	0.013	0.081	NS	X	X	X
Tao.4	212	0.005	0.865	NS			
Tao.4	214	0.070	0.973	NS		X	
Tao.4	215	0.015	0.919	NS		X	X
Tao.4	242	0.061	0.550	NS			
Tao.5	217	0.010	0.928	NS		X	
Tao.5	218	0.028	0.739	NS		X	
Tao.5	219	0.095	0.243	NS	X		
Tao.5	220	0.057	0.523	NS		X	
Tao.5	241	0.174	0.072	NS		X	X
Tao.6	222	0.070	0.550	NS		X	
Tao.6	223	0.122	0.180	NS		X	
Tao.6	224	0.063	0.595	NS	X	X	X
Tao.6	225	0.187	0.054	NS		X	
Tao.6	240	0.187	0.072	NS		X	

Table S5: Octal pathotypes of the isolates used to characterize the resistance spectra of the barley accessions.

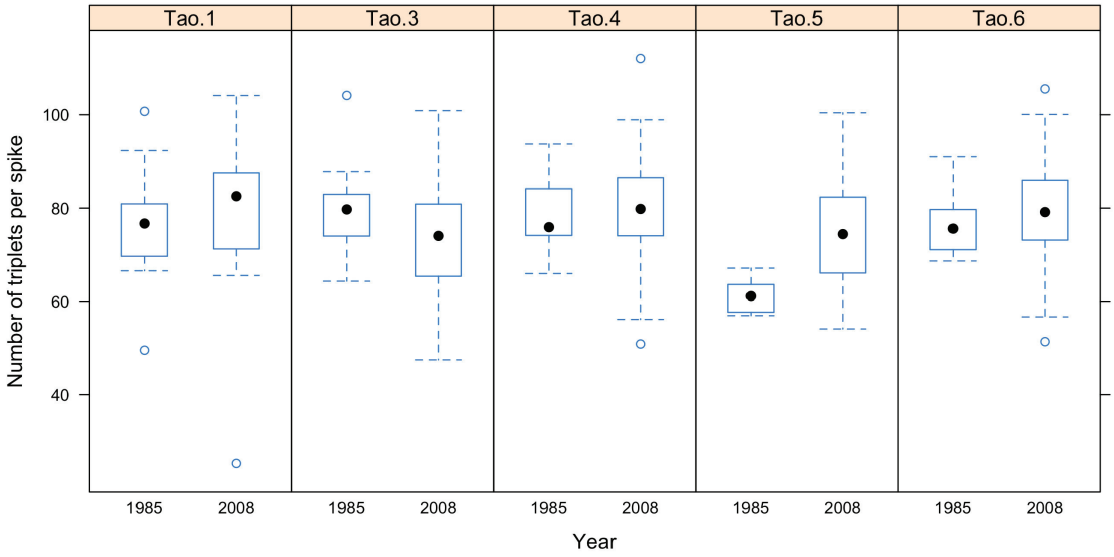
Isolate number	Octal pathotype	Isolate number (cont.)	Octal pathotype (cont.)
1	0004	26	4761
2	0020	27	4773
3	0022	28	4776
4	0023	29	5531
5	0061	30	5625
6	0235	31	5715
7	0323	32	5765
8	0422	33	5771
9	0574	34	5774
10	1002	35	5775
11	1044	36	6040
12	1763	37	6045
13	1765	38	6435
14	2567	39	6535
15	3775	40	6537
16	3777	41	7377
17	4114a	42	7455
18	4114b	43	7555
19	4404	44	7557
20	4417	45	7777
21	4535	46	H-148
22	4553	47	J-462
23	4575	48	S-016
24	4711	49	Y-035
25	4745	50	Y-069

Figure S1: Mean values of three morphological traits: number of triplets per spike (a), spike length (b) and seed length (c), for barley samples collected from the same five sites in both 1985 and 2008. None of the traits were significantly different between years (number of triplets per spike: F=0.611, df=1, P=0.44; spike length: F=0.8212, df=1, P=0.37; and seed length: F=1.9448, df=1, P=0.16).

(a)



(b)



(c)

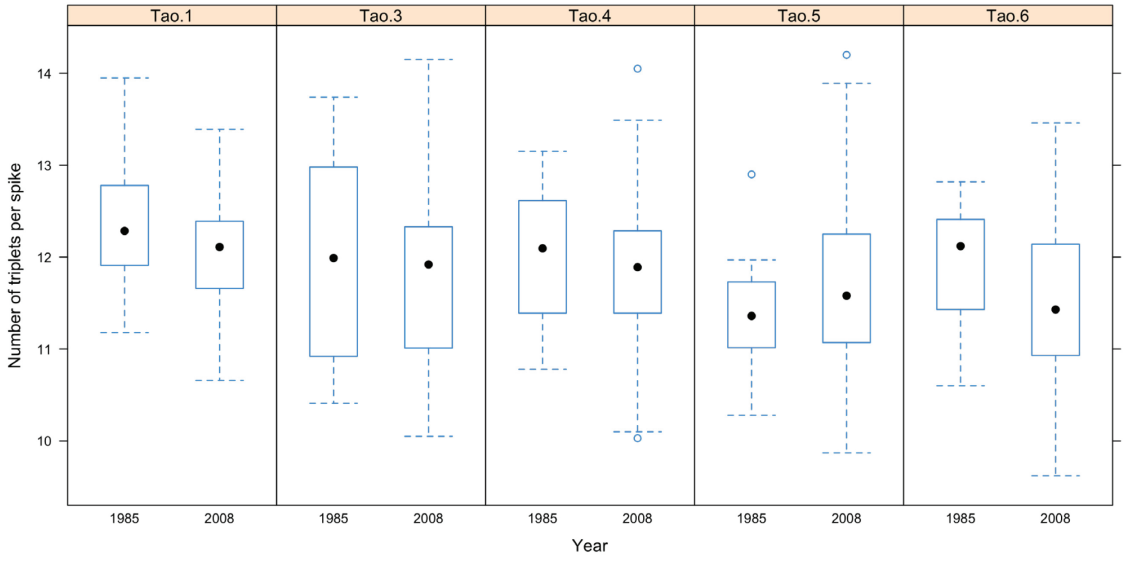

Tao.1
Tao.3
Tao.4
Tao.5
Tao.6
Number of triplets per spike
14
13
12
11
10
1985
2008
Year

Figure S2: Virulence frequencies (+SE) of *Bgh* isolates from the Rabat region and the Taounate region (in the seed collection sites) to the resistance genes and resistance gene combinations in the differential barley varieties.