The evolutionary biology of sex and recombination

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RÉSUMÉ

Certaines suppositions et prédictions reliées à la théorie de la sexualité et de la recombinaison génétique ont été testées. Les résultats ne supportent pas l'hypothèse de la diversification génétique de la progéniture: les espèces de plantes agricoles croisées ne sont pas plus attaquées que celles issues de croisements consanguins; un accroissement de la diversité génétique chez Impatiens en pot ne fut pas accompagné d'un changement de susceptibilité aux parasites; la fréquence de chiasmes n'est pas reliée à la taille des portées chez les mammifères. Par contre, l'idée selon laquelle la sexualité et la recombinaison contribuent à la résistance des jeunes face aux parasites adaptés aux parents ne put être rejetée: les plantes vivaces et les buissons sont plus attaqués que les annuelles; la réduction des dommages aux feuilles chez les jeunes hêtres issus de graines comparée à celle de jeunes plantes issus de stolons décroît avec le temps; le nombre de générations de parasites par génération de l'hôte, ainsi que la fréquence d'excès chiasmatiques sont reliés positivement à l'âge à la maturité chez les mammifères. D'autres sujets, incluant la différence sexuelle de recombinaison, les statistiques de la biologie comparative et la génétique quantitative de la valeur sélective, sont traités.

CONTENTS

PREFACE

It is probably a fairly uncontroversial statement among evolutionary biologists that populations will evolve under natural selection such that the constituent members try to pass on as many copies of their genes to the next generation as possible. However, this is not what sexually reproducing individuals appear to be doing. Consider a diploid female undergoing meiosis, gametogenesis, and fertilization: essentially what she is doing is throwing out half of her genes, investing limited resources in those that remain, and then sharing those resources equally with genes from some male. It is very difficult to see how this sort of altruism could evolve even under very special circumstances, let alone the widespread conditions under which sex exists in nature and seems to be adaptive. The simple alternative of amictic or clonal reproduction would seem to be much more efficient, yet it is far less prevalent, at least among multicellular organisms. This discrepancy between expectation and observation is the paradox of sex (Williams 1975, 1980).

Consider again a diploid female. The genes she decides to keep and around which she produces an egg are not usually either the maternal half of her genes, or the paternal half, but rather some mixture of the two, a mixture which seems to have a strong random component. This mixing is known as recombination, and its main consequence is to break up existing combinations of genes and put together new ones. Now, by definition, more successful or more fit combinations tend to be more prevalent than less fit combinations, and so there would seem to be an inherent bias in the meiotic process to breaking up successful combinations of genes - simply because they are more prevalent - and putting together less successful combinations. This is the paradox of recombination, as once more we have a description of a widespread phenomenon which seems to be at odds with the conventional beliefs of an evolutionary biologist about what sorts of things ought to evolve (Fisher 1930; Turner 1967).

This thesis is a compilation of 8 papers written over the 4 $^1/_2$ years I have been a graduate student working on such problems, presented in chronological order of their conception. While there are a number of ideas in the literature concerning the functions of sex and recombination (Table 1), two of these were seen to be particularly promising, and about half of

Table 1: Some proposed selective forces maintaining sex and recombination (see also Stearns 1987; Michod & Levin 1988).

1) Small genetic correlation for fitness among generations and clumped distribution of offspring.	Williams 1975
2) Environmental change/periodicity	Sturtevant & Mather 1938; Charlesworth 1976; Sasaki & Iwasa 1987
3) Parasites	Jaenike 1978; Hamilton 1982; Rice 1983
4) Resource partitioning by genotype	Bell 1982; Price & Waser 1982
5) Synergistically interacting deleterious mutations	Kondrashov 1982, 1988
6) DNA damage	Bernstein et al. 1985, 1988
7) Directional selection	Maynard Smith 1988

this thesis consists of various tests of these two hypotheses. The first hypothesis notes that an asexual female produces genetically uniform progeny, while a sexual female produces genetically diverse progeny. The latter is said to be better either because the members of a genetically diverse brood will compete less with each other, or because they are less likely to pass on infectious diseases to one another. This is a sibling diversification hypothesis, which may emphasize sib competition, sib contagion, or both. The other hypothesis is in many ways complementary to this one, claiming instead that sex and recombination function to produce progeny which are genetically distinct from their parents. According to this idea, the fitness of a genotype decays as it persists through time due to counter-adaptations by predators, competitors, and in particular parasites, and sex is thought to provide an escape from this process of decline. Thus we have two hypotheses, both claiming that sex and recombination function to create genetic diversity; one emphasizes diversity within generations, the other diversity between generations.

Chapters 2, 3, 6 and 7 describe various tests of the assumptions behind these ideas. Two of these test the predictions of the sib diversification hypothesis that increasing genetic diversity should decrease parasite levels and/or increase yield. In chapter 6 I use agricultural data to compare losses to various pests and pathogens in crops with different breeding systems (U.S.D.A. 1965; Society of Nematologists 1971). Previously, Stevens (1939) had reported that more was written on the diseases of six inbred grains per unit value than on the diseases of three outbred grains, and had concluded that selfers probably suffered more damage (see also Burdon & Marshall 1981). The study reported here is an update and extension of Stevens' work, with better data on crop losses and more of it. In chapter 7 I describe a greenhouse experiment in which the genetic diversity of plants in the same pot was actually manipulated, with both total yield and the levels of two arthropod pests as my response variables (see also Antonovics & Ellstrand 1984; Schmitt & Antonovics 1986; Schmitt & Ehrhardt 1987; Willson et al. 1987; McCall et al. 1989; Tonsor 1989).

In chapter 3 I compare levels of leaf damage on seedlings and suckers of American beech. Both the sib diversification hypothesis and the temporal escape hypothesis predict that sexually produced seedlings should suffer less damage than the asexually produced suckers; in addition, the temporal escape

hypothesis predicts that this advantage to sex should decline with time as the local parasite population evolves - by habitat or natural selection - to get around the host defenses. I test this prediction using height as an index of age. This study follows from various demonstrations of small-scale local adaptation by parasite populations (e.g. Caten 1974; Wainhouse & Howell 1983; Parker 1985; Karban 1989).

The temporal escape hypothesis further suggests that longer lived species should have more problems with parasites than shorter lived species, because of their relatively slower rate of evolution. In chapter 2 I test the assumption behind this idea, that parasite generation times increase less than linearly with host generation time, using data on mammal hosts and their parasitic protozoa and helminths. In chapter 6 I test the prediction that longer lived crops should suffer more damage than shorter lived crops.

The tests decribed up to now have asked whether sex, genetic diversity, and/or parasites act in the way they are purported to by the two hypotheses - they are tests of the hypotheses as theories of ecology. It is also necessary to test them as theories of adaptation - do they predict where we actually see sex and recombination in nature (e.g. Bell 1982; Law & Lewis 1983; Lively 1987; Sharp & Hayman 1988)? To do so I collected information from the literature on rates of recombination in mammals, as measured by chiasma frequencies. The tests of the two hypotheses derive from the opposing predictions they make about how recombination should correlate with certain life history parameters. Sib diversification theories of recombination predict that the more offspring you have, the more recombination you need in order to get sufficient diversification, and so they predict a positive correlation between chiasma frequency and litter size. On the other hand, temporal theories based on the coevolutionary race between hosts and parasites predict that longer lived species will have to recombine more in order to get away from their relatively faster evolving parasites, and so they predict a positive correlation between chiasma frequency and age at maturity. Mammals are a nice group in which to test these two predictions, because litter size and age at maturity are themselves negatively correlated, and so both predictions cannot be correct.

To anticipate, the sib diversification hypothesis fares poorly in the various tests: genetically diverse crop plants were not found to suffer lower levels of pest and parasite damage (chapter 6), increasing the genetic diversity of Impatiens plants in a pot did not increase total production or reduce pest

levels (7), and there is no positive correlation between rates of recombination and litter size in mammals (1). I cannot recommend this hypothesis as a basis for designing interesting and informative studies.

More promising is the idea that sex and recombination function to make progeny different from the parental generation, and so to escape from coevolving parasites. In accordance with this theory, perennial herb and shrub crops suffer more damage than annuals (6), beech seedlings show a transient reduction in leaf parasitism compared to suckers (3), and there are both more parasite generations per host generation and more chiasmata at meiosis in longer lived mammals (1 & 2). This hypothesis seems to be useful in identifying patterns in nature.

As noted above, these tests of the sib diversification and temporal escape hypotheses constitute about half the thesis. The other half includes a miscellany of facts and ideas which I think are at least as interesting, but which do not fall under any narrowly defined heading - hence the very general title. The principle empirical findings are based on compilations of chiasma frequencies. A comparison of rates of recombination in domesticated and wild mammals is presented in chapter 1 - it seems that the former have greatly elevated chiasma frequencies. Male and female chiasma frequencies are compared in chapter 4. One of the stonger empirical generalizations to emerge from the study of genetic systems is that achiasmate meiosis, which has evolved 25-30 times, is always restricted to the heterogametic sex in dioecious species, usually the male (see White 1973; Serrano 1981; Bell 1982; Nokkala & Nokkala 1986; and references therein). The data collected on quantitative sex differences in chiasma frequency do not show any similar trend. These results also bear on ideas proposed by Haldane (1922), Huxley (1928), Trivers (1988) and Bernstein et al. (1988). Also in this chapter I compare chiasma frequencies for species in which only one sex forms cross-overs and species with those in which both sexes form cross-overs, and I compare chiasma frequencies on sex chromosomes and autosomes in female mice.

Biologists have used comparisons among species to test and support various hypotheses at least since Aristotle (Ridley 1983) - indeed this method is used in four chapters of this thesis - but the appropriate statistical methodology is only now being developped (e.g. Harvey & Mace 1982; Ridley 1983; Felsenstein 1985; Bell 1989; Grafen 1989). Such 'comparative methods' are the topic of chapter 5. If one has a dataset of two variables, X

and Y, measured for a number of species, and if more closely related species have more similar values of X and Y than do distantly related species, then a correlation which is statistically significant under the conventional test is likely to arise even if X and Y have evolved completely independently (Raup & Gould 1974). Rather than asking if X and Y are correlated, I suggest that it will often be more interesting to ask if changes in X and changes in Y are correlated, and a method of testing this hypothesis is presented.

In the final chapter I approach the problems of sex and recombination from a theoretical perspective, by developping a quantitative genetic model of fitness. Most previous models of fitness have been constructed to study various aspects of mutational load, and thus have explicitly considered 'the number of deleterious mutations' (e.g. Maynard Smith 1978; Bell 1988a; Kondrashov 1988); less commonly it is 'the number of beneficial mutations' (e.g. Maynard Smith 1978). As an alternative, chapter 8 uses the infinite alleles model commonly used to study stabilizing selection (e.g. Latter 1960, 1970; Kimura 1965; Lande 1976; Bulmer 1980), modified slightly to allow for the facts that mutations and changes in the environment are biased, tending to reduce fitness, and that selection is directional, tending to increase mean fitness. In such a model an advantage to sex and recombination falls out quite readily, at least at the population level, with positive, negative, or even zero epistasis. Whether such a model can account for the short-term advantage of sex observed in Anthoxanthum (Kelley et al. 1988) remains to be seen. Also presented in this chapter is a method of estimating the additive genetic variance of fitness using Fisher's (1930) Fundamental Theorem of natural selection and Mukai et al.'s (1972) data on mutational degradation in Drosophila melanogaster (see also Charlesworth 1987; Rice 1988). Estimates of the genetic variance in fitness are of interest both in their own right and as they relate to 'good genes' theories of sexual selection.

The problems of sex and recombination have attracted a great deal of attention from evolutionary biologists, and it is worth considering why that might be. First, there is the obvious attraction of a paradox, for, as described at the outset, sex and recombination do not seem like the sorts of things which ought to evolve. As well, sex and recombination are widespread in nature, and so there is the potential at least for a very general biological theory to emerge. They would also seem to have rather large effects on fitness, so evolutionary biologists feel they should have something to say about the

matter. Finally, the problems of sex and recombination are as much of interest for highlighting out ignorance about environments and population genetics as they are as problems of adaptation (Bell 1988b). If, as many evolutionary biologists believe, sex and recombination are selected for their genetic consequences, then there is something going on in nature, be it genotype by environment interactions, frequency-dependent selection, high mutational loads, host-parasite cycles, fitness decays through time, or whatever, which is strong enough to pay the twofold cost of sex, plus other costs (e.g. Daly 1978; Lewis 1983), and which we do not know much about.

Most of one's Ph.D. education comes from doing, reading, and talking; this thesis reports what I have been doing, it cites much of what I have read, yet there is little indication of the many educational, stimulating, enjoyable conversations I've had as a graduate student. To the other participants, I am grateful - members of the Bell Laboratory, more generally the McGill Department of Biology, and briefly the Harvey '01 Supergroup' and the Department of Zoology, Oxford. The influence of Graham Bell, my supervisor, will be particularly apparent. Paul Harvey very generously played this role during my 10 month sabbatical. NSERC of Canada provided financial support and Gilbert Cabana translated the abstract.

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All chapters have been or will be submitted for publication in scientific journals; chapters 1, 2, and 5 have already been published and chapter 4 is in press. G. Bell has appeared or will appear as co-author on all except nos. 5 and 8, and P.H. Harvey is also a co-author on chapter 4; in these chapters the first person plural is used. These co-authorships are very much deserved for the advisory, logistic, editorial, and/or financial help Drs. Bell and Harvey have provided - that is, for being good supervisors.

CHAPTER ONE

Mammalian chiasma frequencies as a test of two theories of recombination†

† This chapter started as a term paper for 'The ecology of sex and reproduction' taught by G. Bell. It has been published as: Burt, A. & G. Bell. 1987. Mammalian chiasma frequencies as a test of two theories of recombination. Nature 326:803-805.

A broad survey of asexuality in the animal kingdom is sufficient to reject all theories of sex and recombination except two: the Red Queen and the Tangled Bank[1,2]. The Red Queen theory states that an organism's biotic environment tends to be 'contrary'[3], consistently evolving to the detriment of the organism; sex and recombination result in progeny genetically distinct from their parents and grandparents and thus less susceptible to the antagonistic advances made during the previous generations, particularly by their parasites[1,4,5]. The alternative theory, the Tangled Bank, states that sex and recombination function to diversify the progeny from each other, thus reducing competition between them[1,6]. An extensive survey of mammalian recombination shows that the total number of chiasmata in excess of one per bivalent is strongly correlated with generation time but uncorrelated with fecundity. We conclude that crossing-over may function to combat antagonists with short generation times but does not function to reduce sib competition. Chromosome number is selectively neutral with respect to these factors.

Our test derives from the opposing correlations between rates of recombination and life history parameters predicted by the two theories. According to the Red Queen theory, a coevolutionary model, generation time is an important determinant of selection for sex and recombination: the longer the time between generations, the more contrary will be the environment and the more intense the selection[7-9]. Thus it predicts that recombination is positively correlated with age to maturity (an index of generation time). However, the Tangled Bank is a model of sib competition and selection for sex and recombination will be influenced by fecundity: the larger the number of offspring, the higher the levels of recombination necessary for sufficient diversification[10]. Thus it predicts a positive correlation between recombination and number of offspring. Mammals provide a critical test for the theories because age to maturity correlates negatively with litter size[11] and thus at least one of the specific predictions will be rejected.

The standard measure of recombination is the recombination index RI=n+TC, where n is the haploid number of chromosomes and TC is the total number of chiasmata. In our data set of nondomesticated male

mammals, RI=47 ± 3.2 (mean ± s.e.m., N=32). However, there are a number of constraints preventing either of the two components of this value from being determined solely by selection for recombination. Changes in haploid number: (1) usually have (deleterious) phenotypic effects on the organism[12] which might obscure any effect on recombination; (2) often will not spread through a population simply due to negative heterosis[13,14]; and (3) may act as isolating mechanisms between populations[13,14], resulting in selection that operates between groups rather than individuals and consequently is much weaker[15]. Chiasma frequencies are constrained by the fact that crossing-over is often required for proper segregation of autosomes[16]. Meiotic autosomes lacking a chiasma (univalents) are very rare in mammals (for example, 0.2% for wild Mus musculus[17], 0.05% for Homo sapiens[18]). Furthermore, for all 21 species reviewed in this study for which sufficient data were available, every autosomal bivalent had at least one chiasma, though XY bivalents may have none. These constraints suggest an alternative measure of recombination, the excess chiasma frequency (EC), defined as the number of chiasmata in excess of one, summed across bivalents. This trait does not have the constraints associated with the recombination index, which now can be calculated as RI=2n+EC (assuming the sex bivalent has at least one chiasma). Excess chiasma frequency averages 11.6 ± 1.32 (mean ± s.e.m., N=32) and is independent of haploid number (Table 1). Thus the component of the recombination index most likely to be responsive to selection for recombination is unaffected by the other major component. With this unexpected observation in mind, we now turn to the critical test of the theories, using both recombination index and excess chiasma frequency as measures of recombination.

Complete life history data were available for 24 species. Excess chiasma frequency correlates very well with age to maturity (Tables 1 and 2, Fig. 1). Partial correlation with body weight shows that this association is robust as well as tight: mammals that mature late for their size also have many excess chiasmata for their size (Table 1). Recombination index correlated less well with age to maturity and the association is wholly due to the above relation, as haploid number is independent of age to maturity. Finally, neither excess chiasma frequency nor recombination index correlates positively with litter size.

Table 1. Correlation statistics for measures of recombination and life history in mammals.

Variable (1)	Variable (2)	Partial correlate	Correlation statistic	N	P
EC	n	_	0.001	32	P>0.9
$\log_{10}$MAT	EC	_	0.875	24	P<0.001
$\log_{10}$MAT	EC	$\log_{10}$WT	0.689	24	P<0.001
$\log_{10}$MAT	RI	_	0.471	24	0.05>P>0.02
$\log_{10}$MAT	n	_	0.107	24	0.9>P>0.5
$\log_{10}$LS	EC	_	-0.504*	24	P<0.01
$\log_{10}$LS	RI	_	-0.332*	24	0.05>P>0.01
$\log_{10}$WT	EC	_	0.744	24	P<0.001
$\log_{10}$WT	$\log_{10}$(EC/MAT)	_	-0.675	24	P<0.001
$\log_{10}$WT	EC	$\log_{10}$MAT	0.021	24	P>0.9

Variables as follows: EC, excess chiasma frequency; n, haploid chromosome number; MAT, age at maturity; WT, adult body weight; RI, recombination index; LS, litter size. Correlation statistics are Pearson correlation coefficients, except * which are Kendall's τ; N, no. of species.

Table 2. Least-squares regression statistics for equations of the form $Y=a + bX$ (N=24).

Y	X	a	b	$\bar{X}$	Σx^2	s^2_{YX}
EC	$\log_{10}$MAT(d)	-14.4	10.8	2.5	9.969	15.953
EC	$\log_{10}$MAT(kg)	13.3	5.6	-0.21	26.096	30.458
$\log_{10}$(EC/MAT)	$\log_{10}$WT(kg)	-1.56	-0.30	-0.21	26.096	0.124

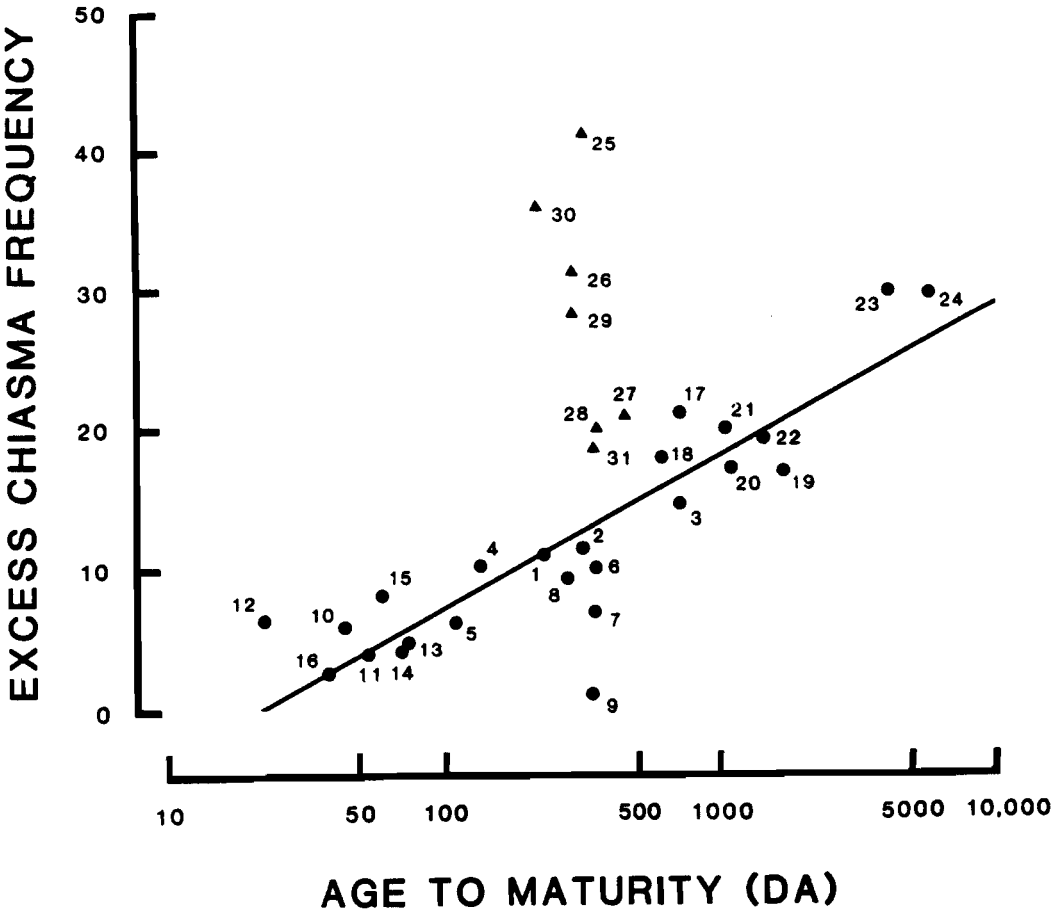

Figure 1. Semilog plot of mammalian excess chiasma frequencies (EC) as a function of age to maturity. Only male mammals were considered. Circles, nondomesticated species; solid line, least squares regression. Triangles, domesticated species not included in the regression. 1, Dasyuroides byrnei; 2, Dasyurus viverrinus; 3, Sarcophilus harrisii; 4, Sminthopsis crassicaudata; 5, Perameles gunnii; 6, Isoodon macrourus; 7,Dasypus novemcinctus; 8, Oryctolagus cuniculus; 9, Thomomys bottae; 10, Cricetus cricetus; 11, Mesocricetus auratus; 12, Lagurus lagurus; 13, Meriones unguiculatus; 14, Apodemus sylvaticus; 15, Rattus norvegicus; 16, Mus musculus; 17, Cebuella pygmaea; 18, Saguinus oedipus; 19, Macaca fuscata; 20, Macaca mulata; 21, Macaca nemestrina; 22, Cynopithecus niger; 23, Pan troglodytes; 24, Homo sapiens; 25, Canis familiaris; 26, Felis catus; 27, Sus scrofa; 28, Bos taurus; 29, Capra hircus; 30, Ovis aries; 31, Equus caballus.

Because age to maturity correlates with body size[19], the allometric equation relating excess chiasma frequency to body weight is also significant (Tables 1 and 2). This observation parallels others that sexuality, broadly defined, tends to increase with increasing body size[1,6,20]. However, recombination per unit time decreases with increasing body size and size has no effect on excess chiasma frequency independently of age to maturity (Table 1). This latter result contradicts one author's prediction of a positive association between body size and recombination[9]; he had reasoned that the evolution of parasites should be faster on larger hosts than on smaller ones.

All the above analyses involve only species that have not been intensively bred; domesticated mammals tend to have much higher excess chiasma frequencies for their age to maturity (residual=16 ± 3.6, mean ± s.e.m., N=7, Fig. 1). This observation parallels a similar finding in cultivated strains of the rye grass Lolium peremme[21]. We suggest that high rates of recombination are indirectly selected in breeding programmes because of their effect in removing negative correlations between desirable characters[22]. Put another way, high recombination is an adaptation to an environment characterized by intense selection in small populations for novel combinations of traits[23], exemplifying a lottery model of selection[24]. Apart from its intrinsic interest, this result also demonstrates that excess chiasma frequencies can change dramatically in only ten thousand years. Thus, excess chiasma frequencies observed at present probably reflect very recent patterns of natural selection and phylogenetic constraints are likely to be minimal.

To conclude, the Red Queen theory has revealed a simple relation which accounts for 75% of the variance in excess chiasma frequency in mammals, although the effect of generation time can be swamped by the intense artificial selection of breeding programmes. The results are also suggestive of a division of labour between chiasmata, one per bivalent to meet the mechanical exigencies of proper segregation and the others for recombination. Finally, chromosome number does not seem to be under selection for its effects on recombination, nor does it affect selection for recombination.

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CHAPTER TWO

Crossovers, parasites, and comparative biology†

† This chapter is a reply to several criticisms of chapter 1. It appears in Nature 330:117-118 (1987).

We have argued[1] that the positive correlation between excess chiasma frequency (EC) and age to maturity among mammals supports the Red Queen theory of recombination, according to which longer-lived hosts must have higher rates of genetic recombination per generation to escape from the counter-adaptations of their parasites. It was an implicit assumption of our test that the number of parasite generations per host generation, and therefore the relative rate of parasite evolution, is positively correlated with host generation time. Greenwood and Koella question this assumption, suggesting that the generation time of parasites may increase isometrically with that of their hosts. Unfortunately, neither author provides any data: we have therefore collated measurements of the prepatent period (time from infection of definitive host to release of first propagules into the external environment) of 37 parasitic species in four major taxa and the age to maturity of their mammalian hosts.

There is indeed a very highly significant positive association between parasite prepatent period and host age to maturity ($p<0.001$), with no indication of heterogeneity of slopes among the four parasite taxa. However, the common slope of this association is much less than unity and so the number of parasite generations per host generation increases steeply with host generation time (Fig. 1). This result is completely consistent with our earlier interpretation and refutes Greenwood's and Koella's conjectures.

We have further argued that the negative correlation between EC and litter size among mammals falsifies the Tangled Bank theory. Greenwood and Koella dispute our rejection, suggesting that longer-lived and less fecund species are subject to more intense competition. This claim, again made without empirical support, misses the basic proposition of the Tangled Bank: that the increased phenotypic variance created by recombination functions to reduce the level of competition within families. It is emphatically a theory of competition between siblings; claims that it does not predict a positive correlation with litter size contradict both common sense and conclusions from computer simulations[2]. Indeed, such a theory clearly does not predict that mammals with litter sizes of one will be among those with the highest levels of recombination, yet this is precisely what we observe. Furthermore, it would appear that litter size is not even a

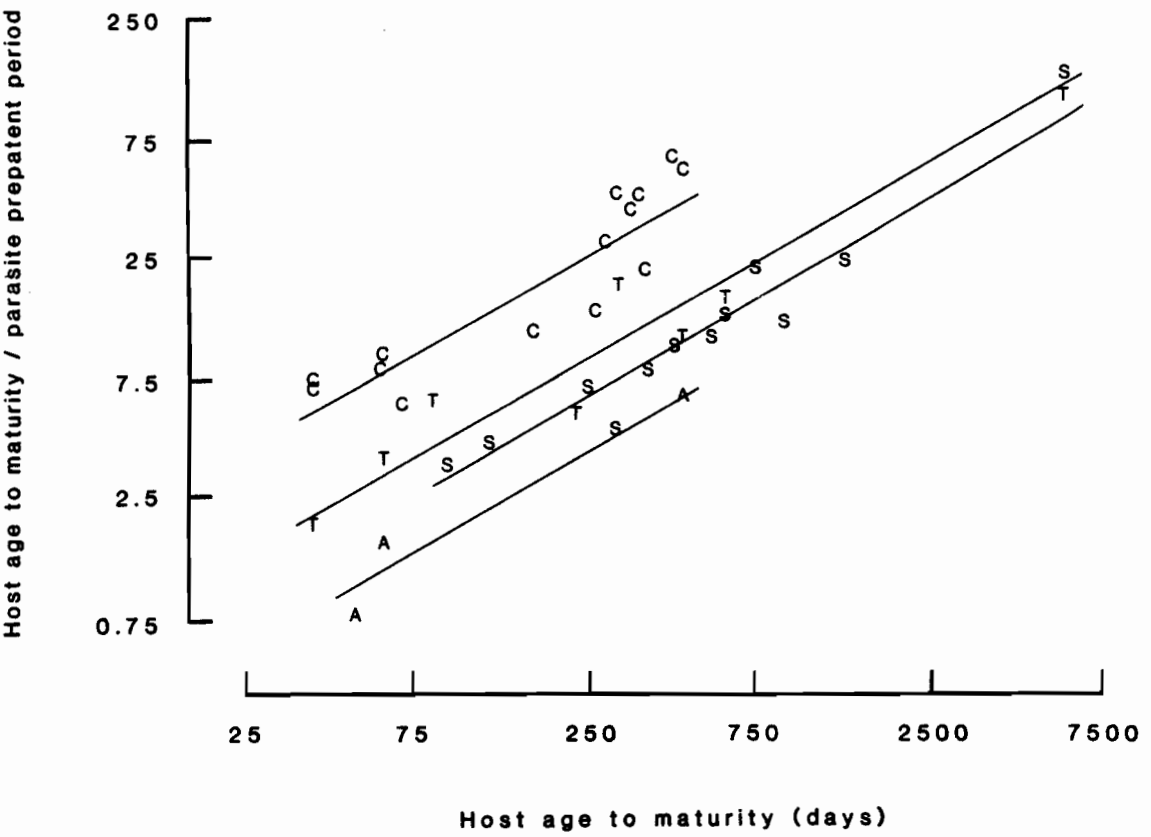

Figure 1. The timescale of mammalian parasites. The ratio of host age to maturity to parasite prepatent period increases with host age to maturity in four parasite taxa with mammalian definitive hosts: A, Acanthocephala[6]; S, Schistosomatidae[7]; T, Cestoda[8]; and C, Coccidia[9] (note logarithmic axes). When a parasite infests more than one species of host, or vice versa, means were used. To quantify the within-taxon association, the data set was transformed such that the four groups had equal bivariate means. The transformed data set has Pearson correlation coefficient r=0.948 (n=37) and reduced major axis slope $v_{Y.X}$=0.867. This latter value is the common slope of the within-taxon association, v, and has standard error about equal to that calculated by analysis of covariance: s_v=0.049. Both major axis regression and analysis of covariance showed the four slopes to be homogeneous (0.9>P>0.5). The four solid lines are drawn with slope v through the (untransformed) bivariate mean of each parasite taxon.

minor contributor to the observed variance in EC: the partial correlataion, after removing the effects of age to maturity, is insignificant ($r= -0.320$, $d.f.=21$, $0.2>P>0.1$). This result seriously damages Greenwood's concluding plea for plurality.

Charlesworth is not interested in saving the Tangled Bank, but rather several theories not considered in our original paper. However, it is not clear why - he offers nothing to counter the well-documented shortcomings of all but one of these theories[3,4]. The author of the remaining theory[5] concludes from simulations that his model cannot account for crossing over in organisms with many chromosomes; 70% of our mammals have haploid numbers greater than any of those used in his simulations ($n>16$).

Finally, Charlesworth claims that our treatment of each species' EC as statistically independent is likely to give spurious positive results. His argument conflates two separate issues. The first, common ancestry and phylogenetic inertia, is unimportant when there exists additive genetic variance for the character under investigation, for the response to selection will be instantaneous on a paleontological timescale. Evidence for such variance in EC comes both from artificial selection and heritability studies[3,4] and from the twofold increase in EC among domesticated mammals[1].Thus the EC of each species is an independent product of natural selection and should be analysed accordingly. Nevertheless, to allay any possible doubts, we have reanalysed our data at the family level; the correlation of family means is almost identical with that of species and the null hypothesis is rejected at the same level that we reported ($r= +0.883$, $d.f.=10$, $P<0.001$).

The second issue is that of confounding selection pressures, for although EC is correlated with age to maturity, it is presumably also correlated with many other characters related to age to maturity. This problem motivated our partial correlation analysis of EC and age to maturity, keeping body weight constant. The latter is strongly correlated with age to maturity among mammals ($r= +0.844$ in our data set), but when the three variables are analysed simultaneously, the partial correlation of EC and age to maturity remains large and significant ($r= +0.689$, $P<0.001$), whereas that of EC and body weight becomes insignificant ($r= +0.021$, $P>0.9$).

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CHAPTER THREE

Seed reproduction is associated with a transient escape from parasite damage in American beech†

† This chapter began as a 2 1/2 day independent project for the 'Ecology and Behaviour Field Course' taught at Mont St. Hilaire.

Simple equilibrium calculations suggest that in organisms which reproduce by both sexual zygote production and asexual vegetative proliferation, the return on reproductive investment in the sexual progeny should be about twice that in the asexual progeny (Williams, 1975). Sexual progeny have been found to produce 40% more inflorescences than asexual progeny in a perennial grass (Kelley et al., 1988; Kelley, 1989). The source of this differential success is a central problem in evolutionary biology, the so-called 'paradox of sex', and is likely to derive from the developmental (Buss, 1987), ecological (Silander, 1985), or genetic (Michod and Levin, 1988) differences between seeds and vegetative propagules. Much recent theory has suggested that seed reproduction may provide a transient escape from parasite attack (Hamilton, 1982; Bell, 1985; Silander, 1985; Bierzychudek, 1987); here we test these ideas by comparing natural levels of parasite-induced leaf damage in seedlings and suckers of American beech (Fagus grandifolia). As predicted by the parasite-escape hypothesis, we find that seedlings suffer less damage than suckers and that this advantage declines with increasing size (and age) of the young tree.

The hypothesized mechanisms by which seedlings may temporarily escape from parasite attack include the developmental, the ecological, and the genetic: reduced parent-offspring transmission of infections, due to developmental isolation during seed formation (Crocker, 1939; Silander, 1985; Bierzychudek, 1987; Parker, 1987); spatial or temporal escape from locally adapted parasites (Caten, 1974; Edmunds and Alstad, 1981; Wainhouse and Howell, 1983; Parker, 1985; Karban, 1989), due to the potentially greater dispersal or longer dormancy of seeds; and increased resistance of novel recombinant progeny, due to time-lagged frequency-dependent selection favouring rare genotypes (Hamilton, 1982; Rice, 1983; Bell, 1985). Despite the variety of proposed selective mechanisms, all predict that seedlings should have reduced levels of parasite damage compared to vegetatively produced offspring, and that this advantage should decay with time as the local parasite population evolves - by natural or habitat selection - to recolonize the host. Testing these predictions provided the impetus for this study.

MATERIALS AND METHODS

American beech is a late-successional, shade-tolerant tree and a major component of the hardwood and mixed forests of east-central North America (Fowells, 1965). It reproduces by both seeds and root suckers, showing considerable geographical variation in the proportion of seedlings and suckers, apparently with increased suckering in the northern end of its range (Held, 1983; Jones and Raynal, 1986). Flowers are monoecious and wind-pollinated; seeds are large (~ 3g), dispersed mostly by gravity and animals, and germinate the year after pollination (Rudolf and Leak, 1974).

The study population is in an undisturbed beech-maple forest at the McGill University Field Station at Mont St. Hilaire, Quebec, Canada. Seedlings and suckers can be distinguished by digging in the soil at the base of the stem and looking for a parental root. We used a paired sampling design, consisting of nearest-neighbour seedlings and suckers (mean distance d=0.98 ± 0.602 (s.d.) m). Leaves were collected from different individuals on three sampling dates: Sept. 1986, July, 1987, and Sept. 1987.

Sampling proceeded by marking transects perpendicular to Lac Hertel on Mont St. Hilaire; in each transect a large tree was chosen as the focus of a search for three nearest-neighbour pairs of seedling and sucker. Twenty leaves were collected from each individual, working down from the topmost stem; only individuals less than 2m in height with the requisite number of leaves were considered. Leaves were brought back to the lab and scored blind (arbitrarily coded, mixed together, scored for damage, and then decoded) on an approximately logarithmic integer scale from 0 (no visible damage) to 6 (over half the leaf eaten or infected). Twelve different transects were sampled on each of three dates. Data were lost for two pairs in the second sample, so the total sample sizes are 106 pairs and 4240 leaves. All leaves were scored by A.B.; the third batch was also scored by G.B. and the correlation between scorers was r=0.818 at the level of leaves, r=0.925 at the level of individual plants. Damage on a sample of leaves from the first batch was also measured using a digital image analyser; the line of best fit relating the proportion of leaf area damaged to our score was: Log(Proportion damaged) = -2.825 + 0.514(Score); r^2=0.851, n=213). Arithmetic mean damages were calculated using this equation and the mean and variance of the log estimates.

RESULTS

The mean leaf damage for all individuals was 12.3 per cent. On a sample of 213 leaves measured using a digital image analyser, over half of this damage (53%) was due to herbivores, recorded as gaps or holes along the edge of the leaf; another 16% was due to rust spots on the surface of the leaf, and the remaining 31% was due to a variety of blemishes and holes on the interior of the leaf. Identifying the species involved was not possible. Suckers suffered 1.1 times more damage than seedlings (12.9 and 11.8 per cent respectively; Fig. 1). This difference between seedlings and suckers does not differ significantly between the three samples ($F_{2,103}$ = 0.37, n.s.). Analysed separately, all three samples show the same trend, though the effect is statistically significant ($p<0.05$) in only one. All three also show a significant interaction between mode of reproduction and pair, indicating that the advantage of seed reproduction differs between pairs; the next analysis deals in part with this unexplained variance.

To test whether the advantage of seed reproduction is transient, we estimated the correlation between damage and height separately for both seedlings and suckers. The results are as predicted by the parasite-escape hypothesis: the slope of the relation in the seedlings is both significantly greater than zero and significantly greater than in the suckers (0.57 ± 0.163 (s.e.) vs 0.03 ± 0.160, $p<0.01$ (one-tailed); Fig. 2). The seedling intercept is also significantly lower than the sucker intercept (1.99 ± 0.196 vs 2.77 ± 0.189 $p<0.01$). One gets the same results by controlling for main effect differences between samples: damage is significantly correlated with height in seedlings ($p<0.001$), but not in suckers ($p>0.5$) and the common slopes of seedlings and suckers differ significantly ($p<0.01$). Again, the three samples analysed separately show the same trends: seedling damage is positively correlated with height in all three samples, significantly so in two of them, and the slope of damage on height is greater among the seedlings than the suckers in all three samples, significantly so in one of them. Only two of the three correlations between sucker damage and height are positive, and none are significant. We also measured age directly by counting growth scars and age rings, and these lead to even more highly significant differences between seedlings and suckers in the predicted

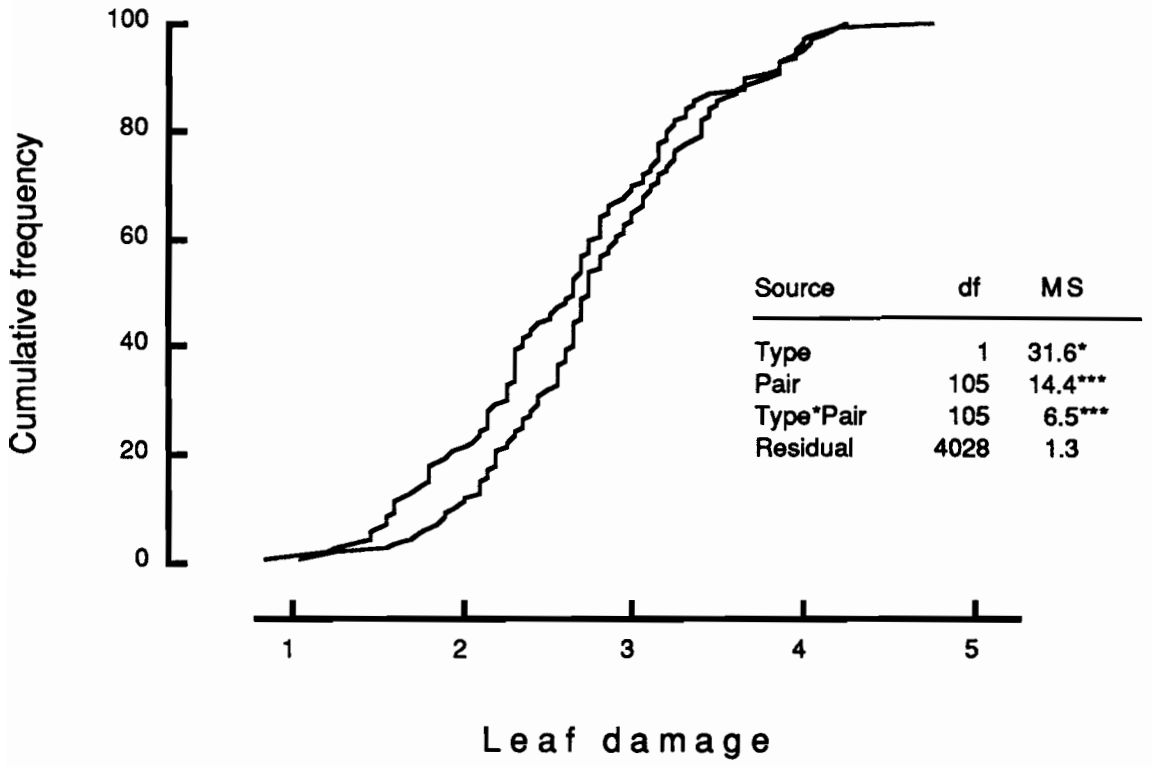

Figure 1. Cumulative frequency distribution of leaf damage for seedlings (upper) and suckers (lower) of American beech. Damage calculated as means for 20 leaves. Also shown is the mixed-model analysis of variance for leaf damage.

* - $p<0.02$ (one-tailed); *** - $p<0.001$ (two-tailed)

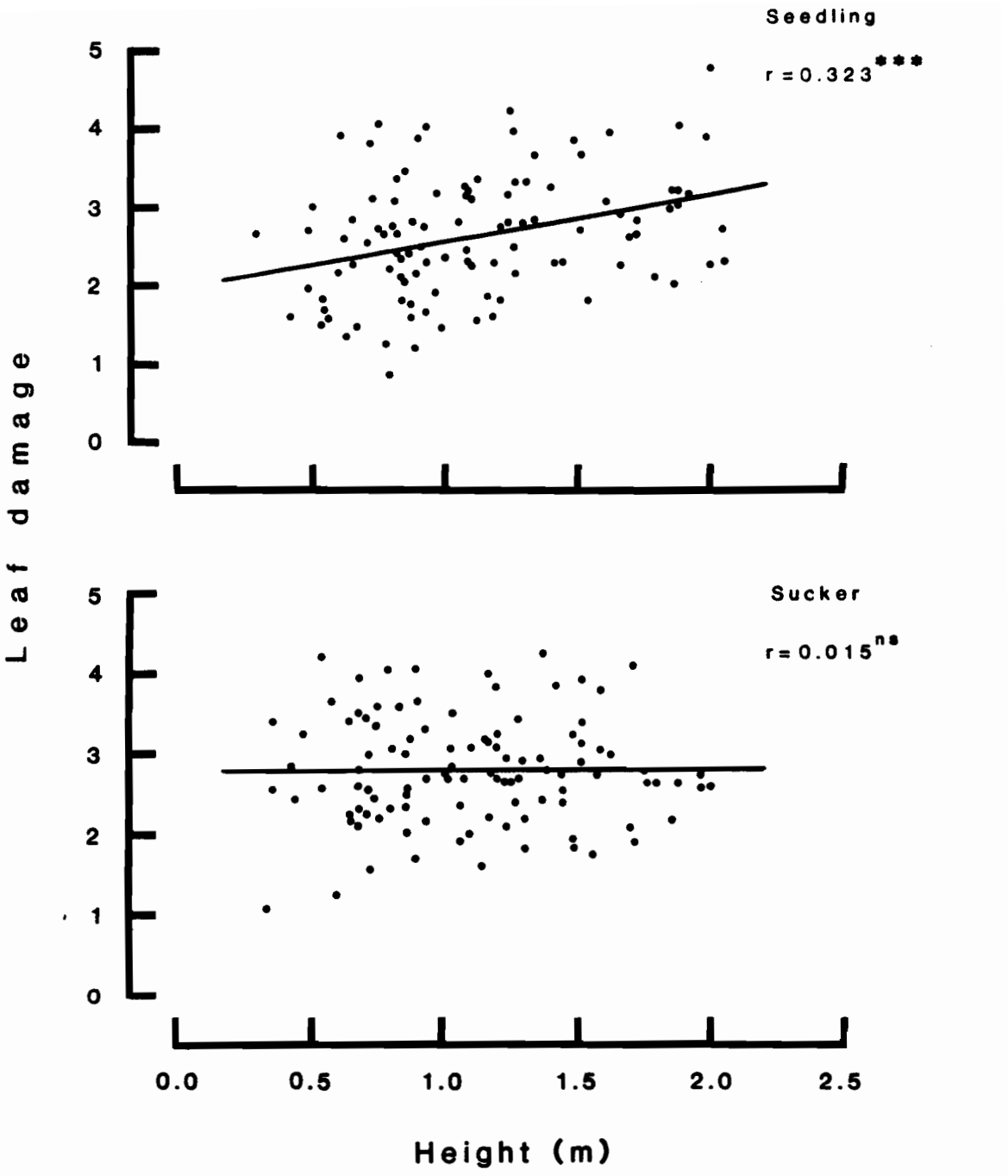

Figure 2: Leaf damage as a function of height in seedlings and suckers of American beech. Each point is an individual, with damage scores calculated as means of 20 leaves.
***-p<0.001.

direction; however, because suckers grow faster than seedlings and the sampling design excluded plants outside a certain height range, not age range, we have chosen to base our interpretation on the relationships with size rather than age.

Note that this difference in slopes indicates that the magnitude (and statistical significance) of the difference between seedling and sucker damage depends on the height of the trees concerned. For example, at 0.5m, the lower end of our sampled range, suckers have 1.7 times more damage than seedlings (12.7 vs 7.4 per cent; $p<0.0025$, one-tailed). At 2m, the upper end of our range, the difference is no longer significant.

DISCUSSION

The two major predictions of the parasite-escape hypothesis are supported: seedlings suffer less damage than vegetatively produced individuals, and this advantage decays with time. Though our study was not specifically designed to distinguish the potential selective mechanisms underlying the escape from parasitism, two in particular seem unlikely to account for our observations. First, most of the observed damage was due to herbivores, we did not find any evidence in the remainder of a systemic disease, and there is no mention of systemic foliage diseases in a review of beech pathology (Hepting, 1971); this suggests that the developmental hypothesis is perhaps an unlikely explanation. Second, beech trees have no extended dormant period or seed bank; thus temporal escape is not possible. We are not aware of any similar reasons for discounting the other two proposed mechanisms, that seeds allow escape in space from locally adapted parasites, or genetic escape from parasites adapted to previous generations.

It is not possible to relate the observed differences in leaf damage to the predicted two-fold difference in return on reproductive investment, both because sexual and asexual reproduction involve quite different costs (flowers and seeds versus roots and nutrients) and because the relationship between leaf damage and individual fitness is unknown. Two points seem worth making. First, while leaf damage may be the most visible and the most easily measured form of damage, stem and root parasites may be equally important determinants of host fitness. If seedlings also suffer less

from these sources, then the advantage of seed reproduction will be compounded. Second, even if all else were equal, the mean annual survival of seedlings need only be 1.017 times greater than that of suckers in order to result in a two-fold differences at the end of a 40 year juvenile period (Fowells, 1965). This difference would be all but impossible to observe directly. Fortunately, the data presented here suggest that much of this difference may be concentrated in the first metre of life.

ACKNOWLEDGEMENTS

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CHAPTER FOUR

Sex differences in recombination†

† This chapter will appear as: Burt, A., G. Bell & P.H. Harvey. 1990. Sex differences in recombination. (J. Evol. Biol. in press).

ABSTRACT

One of the stronger empirical generalizations to emerge from the study of genetic systems is that achiasmate meiosis, which has evolved 25-30 times, is always restricted to the heterogametic sex in dioecious species, usually the male. Here we collate data on quantitative sex differences in chiasma frequency from 54 species (4 hermaphroditic flatworms, 18 dioecious insects and vertebrates and 32 hermaphroditic plants) to test whether similar trends hold. Though significant sex differences have been observed within many species, only the Liliaceae show a significant sexual dimorphism in chiasma frequency across species, with more crossing over in embryo mother cells than in pollen mother cells; chiasma frequencies are unrelated to sex and gamety in all other higher taxa studied. Further, the magnitude of sexual dimorphism, independent of sign, does not differ among the three main ecological groups (dioecious animals, plants, and hermaphroditic animals), contrary to what would be expected if it reflected sex-specific selection on recombination. These results indicate that the strong trends for achiasmate meiosis do not apply to quantitative sex differences in recombination, and contradict theories of sex-specific costs and benefits. An alternative hypothesis suggests that sex differences may be more-or-less neutral, selection determining only the mean rate of recombination. While male and female chiasma frequencies are more similar than would be expected under complete neutrality, a less absolute form of the hypothesis is more difficult to falsify. In female mice the sex bivalent has more chiasmata for its length than the autosomes, perhaps compensating for the absence of recombination in males. Finally, we observe that chiasma frequencies in males and females are positively correlated across species, validating the use of only one sex in comparative studies of recombination.

Observations of sex differences in the amount of recombination at meiosis are common, even among autosomal genes, and date back to the early days of genetics (Morgan, 1912, 1914; Haldane, 1920). These differences can be usefully divided into three types, according to their cytogenetics. First, both sexes may have normal chiasmate meiosis, but with quantitative differences in the number or position of cross-overs (e.g. mice). Second, one sex may have an achiasmate meiosis, with no crossing-over of homologous chromosomes at all (e.g. male fruit flies). Finally, there may be neither independent segregation of nonhomologous chromosomes nor crossing-over in one sex (always the male), as in haplodiploid and parahaplodiploid species (e.g. bees, scale insects). Here, we will be mainly concerned with the evolution of quantitative sex differences in recombination.

Haldane (1922) gave the first general treatment of the problem, advancing the empirical claim that recombination tends to be reduced in the heterogametic sex. Huxley (1928) similarly suggested that whenever a marked sex difference in recombination occurred, it was always the heterogametic sex that had the lower value. Both authors proposed the same explanation: if gender is determined by two or more loci on the sex chromosomes, then selection against intersexes will favour reduced recombination between these chromosomes in the heterogametic sex, and as a pleiotropic effect the recombination of autosomal chromosomes may also be reduced.

These views have been questioned on occasion, both because there are some exceptions to the empirical generalization (e.g. Dunn and Bennett, 1967; Callan and Perry, 1977) and because the proposed explanation cannot account for observed sex differences in hermaphrodites (e.g. Ved Brat, 1966). However, there was no alternative theoretical perspective until Trivers (1988) recently revived the subject, with slightly different empirical claims and a provocative new explanation. According to Trivers, recombination tends to be lower in males than females, as well as lower in the heterogametic sex than the homogametic sex, though he acknowledges that there are many exceptions to these rules. Trivers suggests that both reduced recombination and heterogamety are consequences of selection

being more intense in one sex (usually the male) than the other. He argues that reproducing individuals of the sex experiencing more intense selection will, on average, have better combinations of genes than those of the other sex, and so the cost of breaking up those combinations should be higher. Bernstein et al. (1988) counter with an alternative explanation, that rates of recombination tend to be higher in females because oogenesis is associated with higher metabolic rates, and thus more DNA damage, than spermatogenesis; however, they admit to being puzzled by the association with gamety.

Most other theories of recombination can be adapted to predicting sex-specific optimal recombination rates. For example, many theorists believe that the main function of recombination is to reduce linkage disequilibrium (e.g. Felsenstein, 1988; Maynard Smith, 1988; Kondrashov, 1988). As two potentially important sources of linkage disequilibrium are selection and drift, one might expect that the sex experiencing the more intense selection, or otherwise having the higher variance in reproductive success, should have more recombination. This prediction is exactly opposite to that made by Trivers. Other predictions follow from the various proposed diversity theories of recombination (Williams, 1975; Bell, 1982; Tooby, 1982). Alternatively, sex differences in recombination may be more-or-less invisible to natural selection, the latter determining only the mean value. Simulations by Nei (1969) indicate that sex differences in recombination may have very little effect on population mean fitness.

Information currently available on achiasmate meiosis in no way contradicts Haldane's, Huxley's, and Trivers' empirical claims: we know of 25-30 independent origins of achiasmate meiosis among dioecious animals (A. Burt, unpublished; see Serrano, 1981; Bell, 1982; Nokkala and Nokkala, 1986 and references therein) and every time it has evolved in the heterogametic sex, which all but twice is the male (exceptions are Copepoda and Lepidoptera/Trichoptera). Here, we bring together the available data on quantitative sex differences in chiasma frequencies, to further test the strength of the proposed trends and, if possible, to test the various explanations. To this end we also examine the magnitude of sexual dimorphism in chiasma frequency, independent of sign, and look for evidence of compensation between the sexes.

One further motivation for this study is to estimate the correlation between male and female chiasma frequencies across species, thus determining whether the value for one sex is a good indicator of what is happening in the other sex and in the species as a whole. This estimate is important because male meiosis is usually more easily studied than female meiosis, and so comparative surveys of chiasma frequencies tend to only use data for males (e.g. Burt and Bell, 1987; Sharp and Hayman, 1988). There are about 20 times more chiasma frequencies for males in the literature as for females.

DATA AND ANALYSIS

Rates of recombination can be measured both by counting chiasmata through the microscope and by crossing marked individuals to construct a linkage map. Counts of chiasmata are available for many more species than are extensive linkage maps, and here we will restrict ourselves to the former. As with any comparative analysis using data from the literature, the quality of estimates varies - for example, in techniques and sample sizes. Actually counting chiasmata in some species is quite straight-forward and in others quite difficult; female mammals are notoriously difficult. For plants, often only metaphase figures are available, whereas counts at the earlier diplotene stage are usually considered more accurate. Perhaps more importantly, the methods used are often different for the two sexes, so that observed differences between males and females may be due to differences of technique rather than real. This problem is particularly acute when the data for the two sexes come from different studies (3 of 6 amphibians and 2 of 4 mammals in our data set).

Perhaps the best measure of recombination to be got from a meiotic spread is the proportion of the genome which recombined. This value can be calculated by measuring the distances between the ends of chromosomes and the nearest chiasma and between neighbouring chiasmata, and expressing these as a proportion of the total genome length. For n bivalents and C chiasmata, there will be n+C such distances, d_i. The proportion of the genome which recombines is then equal to the proportion of pairs of loci which are on different segments: $P=1-\sum d_i^2$. This value will be a function of the number and size distribution of chromosomes and the

number and position of cross overs. Corrections could be made for obviously noncoding fractions of the genome simply by not including them in the calculations.

Unfortunately, this proportion has yet to be reported for any species. Instead, we shall use simple counts of the number of chiasmata, noting that for any given distribution of cross overs in the genome, our measure P increases monotonically with the number of chiasmata. Ignoring possible sex differences in the position of cross overs will lead to some inaccuracy: for example, Fletcher & Hewitt (1980) observe that males of Chrysochraon dispar have slightly more chiasmata per bivalent than females, but that they are terminalized to such an extent that the effective amount of recombination is greater in females. However, we believe that the error introduced will be negligible.

One possible check on the data is to compare sex differences in chiasma frequency and linkage map lengths. Unfortunately, we know of map length data for only three species in our data set, all mammals: Sminthopsis crassicaudata (Bennett et al., 1986), mice (Dunn and Bennett, 1967), and humans (Donis-Keller et al., 1987). For S. crassicaudata and mice the sex differences in chiasma frequency and map lengths are in the same direction, but not for humans: the cytogenetic data suggest that males have more chiasmata than females (51 vs 43; Lange et al., 1975; Jagiello et al., 1976), but the genetic data indicates they have shorter map lengths (2017 vs 3857cM; Donis-Keller et al., 1987). Apparently, the female chiasma frequencies are greatly underestimated. This corroborates Chandley's (1988:20) statement that, due to technical difficulties, "accurate counts of chiasmata for the human female still remain to be established." As the problems of getting sufficient appropriate material (oocytes at time of ovulation) are much greater for human females than for other species, this discrepancy is unlikely to be representative of the rest of the data. Indeed, among other organisms for which both chiasma frequencies and extensive genetic maps exist, there is a strong correlation between the two ($r=0.85$, $n=10$; A. Burt, unpublished). Here, we have excluded humans from further analysis.

Having decided to use counts of chiasmata at meiosis, there still remains a number of possible indices of recombination. Burt and Bell (1987) defined the excess chiasma frequency as the number of chiasmata

per bivalent in excess of one, summed across bivalents. This measure was considered to most accurately reflect selection for recombination, independently of the various constraints on changes in chromosome number and the mechanical role of chiasmata in proper segregation. However, it does not make much biological sense for polyploid and achiasmate species, both of which are represented in our data set. Therefore we use here the number of chiasmata per autosomal bivalent. Interpretations are also made easier by this choice, since in our data set the chiasma frequency per bivalent is independent of chromosome number ($r=-0.105$, $n=54$, $p>0.4$), while excess chiasma frequency is positively correlated with chromosome number ($r=0.317$, $n=54$, $p<0.02$). In any case, choice of index does not affect the conclusions drawn.

Data are available for 54 species of animals and higher plants (Appendix), approximately 0.002% of all known animals and higher plants. Unfortunately, the data set is taxonomically unrepresentative: there are 8 species of acridid grasshoppers, but no other arthropods; 4 Triturus newts, but no fish, reptiles, or birds; 22 species in the Liliaceae, but only two dicots. This nonrandomness means that we cannot put much weight on overall trends and must instead look within lower taxa: since we cannot make definitive statements about all animals and higher plants, we shall try to say something about acridid grasshoppers, Triturus, and the Liliaceae.

RESULTS

Correlations. Across all chiasmate species there is a positive correlation between male and female chiasmata per bivalent ($r=0.75$, $n=54$, $p<0.001$; Fig. 1). This result seems to be fairly robust, as the sign of the correlation is positive in 9 of 11 independent taxa (Table 1). The exceptions are amphibians and Oedipodinae, a subfamily of grasshoppers, though neither are significantly negative.

Sexual dimorphism. Across all species females seem to have more chiasmata than males (paired t-test, $t=2.49$, $n=54$, $p<0.02$). Closer examination of the data shows that this trend holds for Lilium (all 8 species, $p\sim0.008$) and probably Liliaceae genera (all 5 genera have more species with more chiasmata in the female than the male, $p=0.0625$).

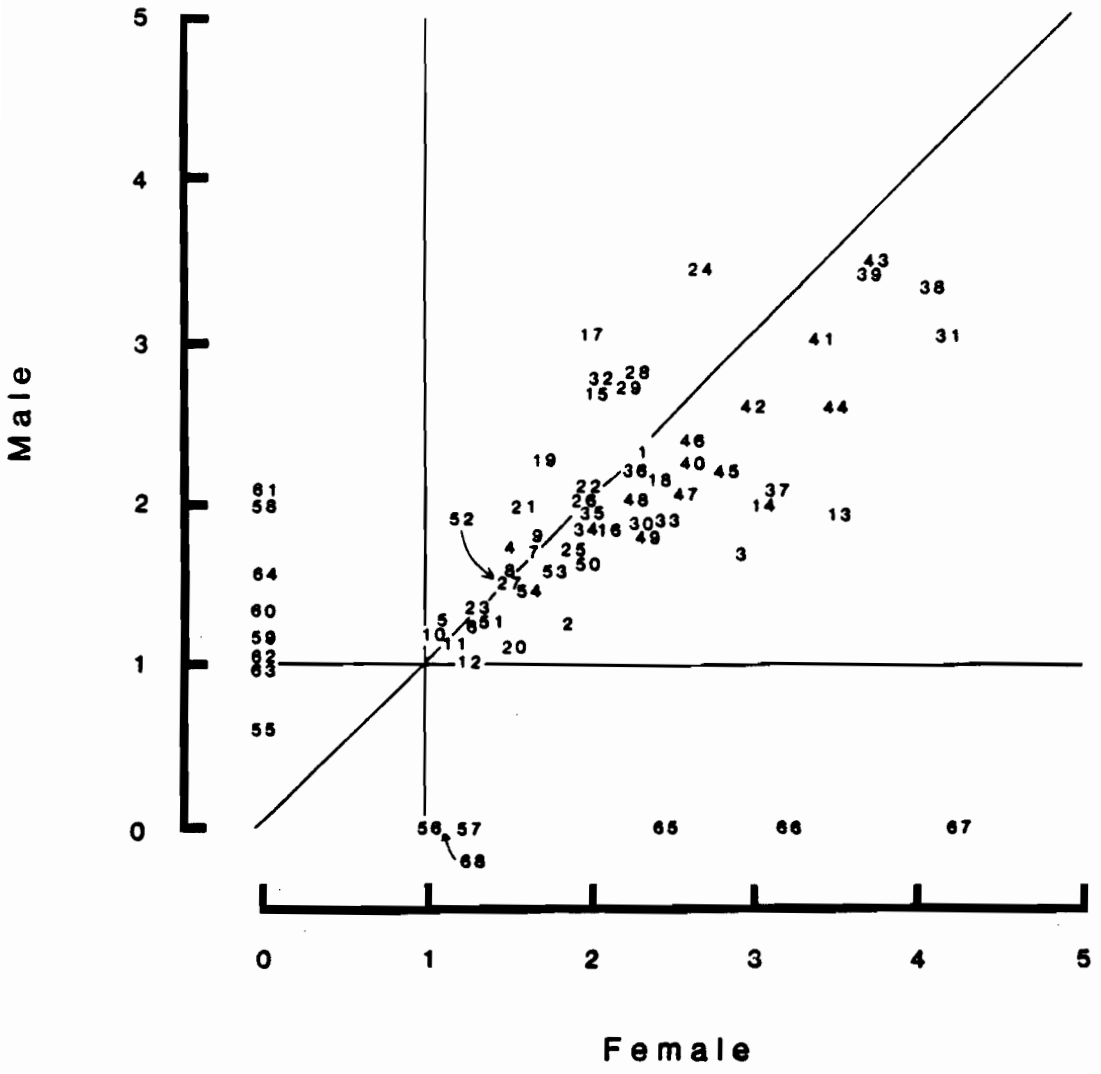

Figure 1: Male vs female chiasma frequencies per bivalent with line of equality. Numbers refer to species in the appendix. Note the large gap separating chiasmate and achiasmate species: all chiasmate species have at least one chiasmata per bivalent (horizontal and vertical lines).

Table 1. Chiasma frequencies per bivalent for males and females. Lettered entries are phylogenetically independent (Burt, 1989). n is the number of species; t refers to paired t-tests; |Diff| is the average magnitude of sexual dimorphism, the mean of the absolute value of the difference between male and female; r is the correlation coefficient for male and female values. *- $p<0.05$; **- $p<0.01$; ***- $p<0.001$.

	Taxon	n	Xta/bivalent		t	|Diff|	r
			Male	Female			
	All	54	2.05	2.23	2.49*	0.41	0.75***
	Animalia	22	1.77	1.87	0.76	0.42	0.49*
	Platyhelminthes	4	1.74	2.14	1.23	0.52	0.25
a	Trematoda	1	2.31	2.31	---	0.00	---
b	Turbellaria	3	1.56	2.09	1.27	0.69	0.20
	Insecta, Orthoptera, Acrididae	8	1.37	1.33	0.71	0.11	0.89**
c	Eyprepocnemidinae	1	1.28	1.09	---	0.19	---
d	Melanoplinae	1	1.23	1.27	---	0.05	---
e	Gomphocerinae	3	1.69	1.60	3.25	0.09	0.94
f	Oedipodinae	3	1.11	1.16	0.42	0.14	-0.98
	Chordata	10	2.11	2.19	0.33	0.64	0.04
	Amphibia	6	2.27	2.52	0.61	0.81	-0.57
g	Anura	1	1.94	3.52	---	1.58	---
	Urodela	5	2.34	2.32	0.05	0.66	-0.52
h	Salamandra	1	2.00	3.07	---	1.07	---
i	Triturus	4	2.42	2.14	0.89	0.56	-0.50
	Mammalia	4	1.86	1.70	0.75	0.37	0.59
j	Marsupialia	1	2.27	1.70	---	0.57	---
k	Rodentia	1	1.10	1.52	---	0.42	---
l	Primates	2	2.03	1.78	1.67	0.25	1.00
	Plantae, Angiospermae	32	2.25	2.48	2.88**	0.39	0.82***
m	Dicotyledonae, Leguminosae	2	2.38	2.00	1.03	0.39	1.00
	Monocotyledonae	30	2.24	2.51	3.50**	0.39	0.84***
n	Commelinaceae	1	1.70	1.90	---	0.20	---
o	Gramineae	2	1.76	1.74	2.00	0.02	1.00
	Liliaceae	22	2.47	2.81	3.30**	0.49	0.73***
p	Allium	8	2.36	2.44	0.38	0.52	0.50
q	Lilium	8	2.87	3.36	5.93***	0.50	0.89**
r	Tulbaghia	4	2.08	2.47	4.53*	0.39	0.70
s	Orchidaceae	5	1.50	1.63	1.78	0.15	0.84

However, there is no evidence that the trend applies to other plant taxa or any animal taxon (Table 1). As many species individually show significant sex differences in chiasma frequency (Appendix), this result indicates that there is a large sex x species interaction effect. All dioecious species in the data set are male heterogametic (except one species with unknown sex chromosome system), so the absence of a consistent sex difference also indicates that there is no consistent difference between homo- and heterogametic sexes.

Ranges. The magnitude of sexual dimorphism, independent of sign, is given by |male-female|. This is also the range, a measure of dispersion. The idea of sex-specific optima suggests that the magnitude of sexual dimorphism should be correlated with the opportunity for sex differences in selection, and thus presumably in the order

dioecious animals	>	hermaphroditic plants	>	hermaphroditic animals

.

Mean ranges for these groups are 0.40 ± 0.102 (s.e.), 0.39 ± 0.055 and 0.52 ± 0.269 chiasmata/bivalent respectively (Table 1); there is no significant difference among groups ($F_{2,51}$=0.20), contradicting this prediction.

We can also test the idea that sex differences in recombination are neutral. In its strongest form, this hypothesis predicts that species will drift up and down lines of neutral equilibrium representing isoclines of equal total recombination. The expected magnitude of sex differences can be calculated under this model as follows. Since bivalents are constrained to having at least one chiasma for proper segregation, we shall consider the number of chiasmata per bivalent minus one (i.e. the mean number of 'excess chiasmata' per bivalent). As both male and female excess chiasma frequencies are non-negative, the range is constrained mathematically to being in the interval [0, 2m], where m is the mean of male and female values. The neutral hypothesis claims that all values within this interval are equally likely, and thus that the expectation of the range is equal to m. In figure 2 we show the range of excess chiasmata per bivalent versus the mean. Almost all points fall below the line of equality, indicating that the male and female values are more similar than predicted.

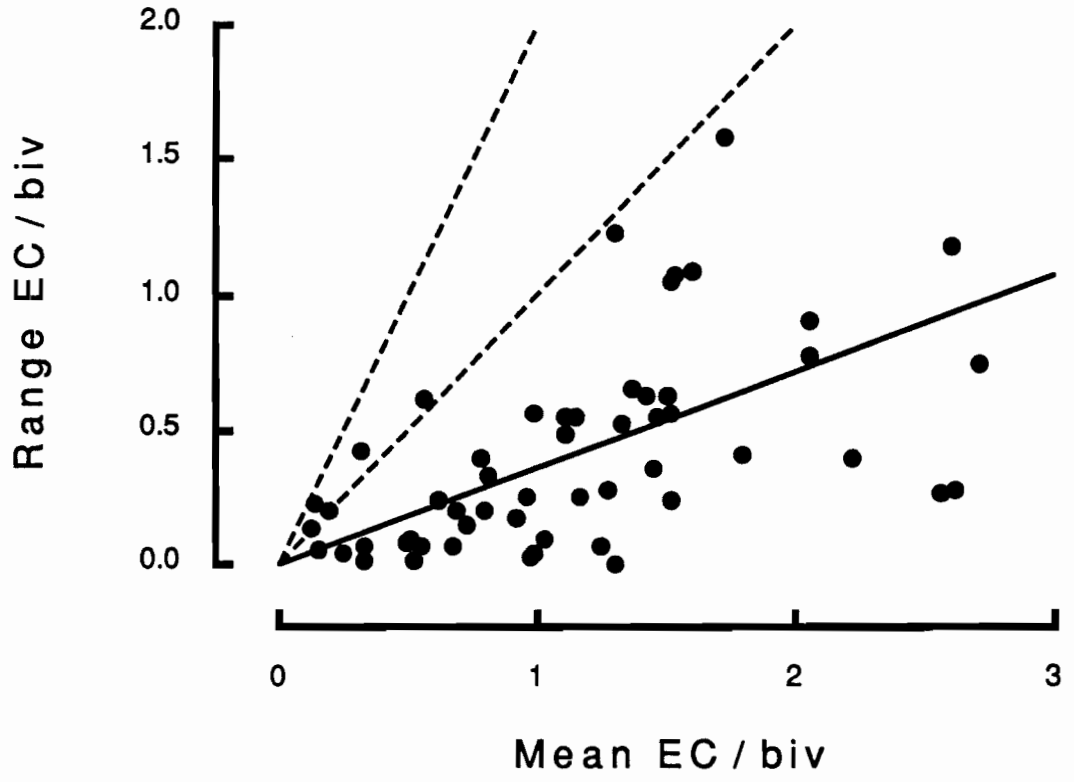

Figure 2: Range of excess chiasmata per bivalent between sexes versus the mean. Points are mathematically constrained to fall below the top dashed line (y=2x), and are expected by the neutral hypothesis to fall around the lower dashed line (y=x). Solid line is the weighted regression fitted through the origin and the bivariate mean (y=0.36x).

Compensation. If selection determines only the mean rate of recombination, then the optimal rate for one sex will depend on what the other is doing, and vice versa. We test for evidence of such tradeoffs in three situations: achiasmate species, haplodiploid species, and the sex chromosomes.

In species where one sex has an achiasmate meiosis, one might expect the other to have a higher than average chiasma frequency to compensate. Chiasma frequencies for species in which one sex is achiasmate are available for 13 species (Appendix). The rate of recombination in the chiasmate sex is to be compared to that of the same sex of a fully chiasmate species. Note that for two such comparisons to be independent, they must involve different parts of the phylogeny - a comparison between a Lepidopteran and an Orthopteran is not independent of a comparison between another Lepidopteran and another Orthopteran (see Burt, 1989 for discussion). In our data set there are three such 'phylogenetically independent' comparisons between an achiasmate species and a fully chiasmate species: Neorhabdocoela (Turbellaria, nos. 55 vs 3 in Fig. 1), Insecta (56-64 vs 5-12) and Fritillaria (Liliaceae, 65-67 vs 37). In no case is there any indication of compensation.

Data are also available for one haplodiploid species, the parasitic wasp Aphytis mytilaspidis (Rossler and DeBach, 1973, no. 68 in Fig. 1). Again there is no indication of compensation compared to wholly sexual insects (Fig. 1).

Finally, in species with strongly dimorphic sex chromosomes the X (or Z) has a haplodiploid mode of inheritance and one can test for compensation by comparing chiasma frequencies of the sex bivalent to those of autosomes in the homogametic sex. The only data available on chiasma frequencies for individually identifiable bivalents in the homogametic sex are for mice (Jagiello and Fang, 1987). In figure 3 we plot chiasma frequency as a function of chromosome length and observe that the X bivalent has significantly more chiasmata at meiosis than the autosomes ($t=3.4$, $p<0.01$). This observation supports the notion of compensation.

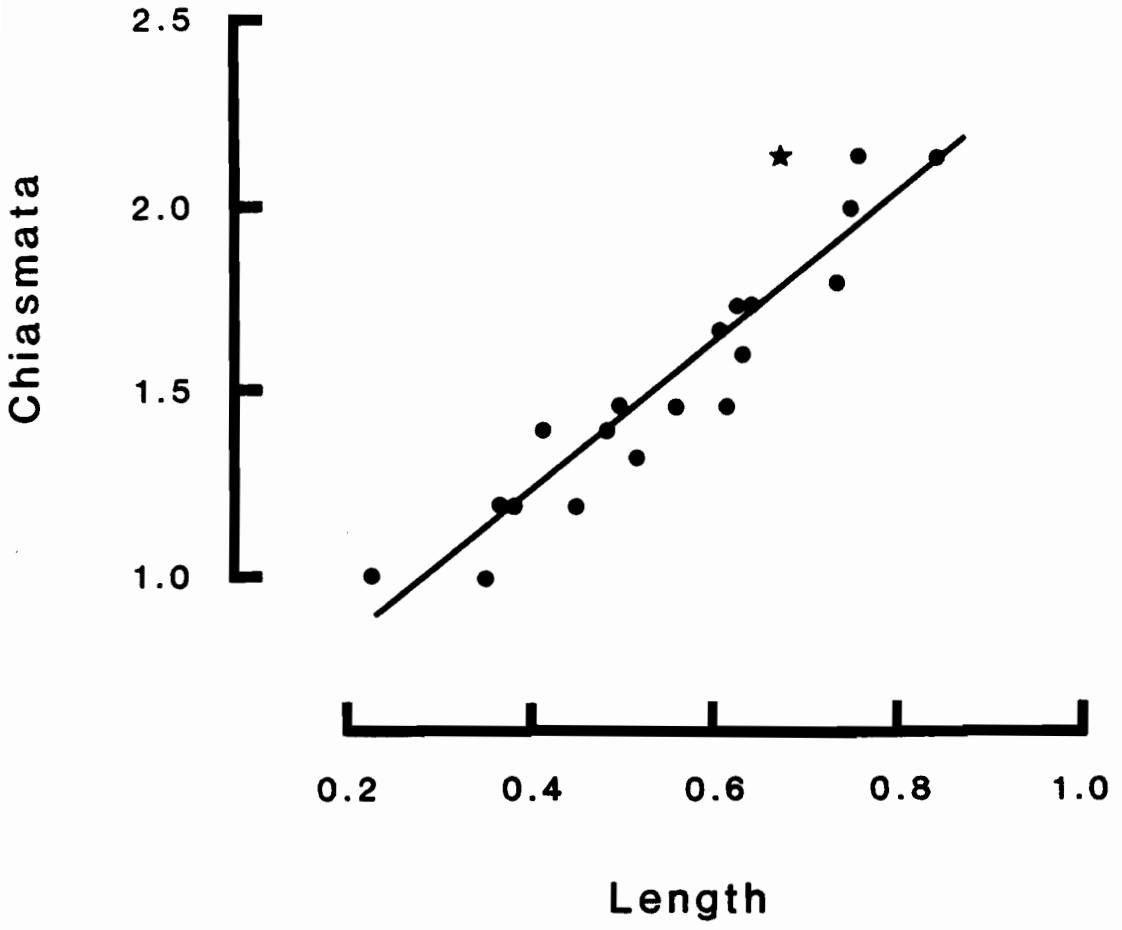

Figure 3: Chiasma frequency as a function of chromosome length (arbitrary units) in female mice. Regression line is for autosomes only (circles; r=0.95). The sex bivalent (star) has significantly more chiasmata for its length than the autosomes. Chromosome length (from idiograms of oocyte chromomeres) and chiasma frequiencies (mean of 15 oocytes) from Jagiello & Fang (1987).

DISCUSSION

The strong empirical generalizations for the occurrence of achiasmate meiosis in dioecious species do not hold for quantitative sex differences in chiasma frequency: chiasma frequencies do not differ consistently between homo- and heterogametic sexes, nor between males and females, outside the Liliaceae. Dunn and Bennett (1967) come to similar conclusions, based on many fewer species, in their review of sex differences in genetic map lengths. These results suggest that the two types of sex differences require different explanations.

Early accounts of sexually dimorphic rates of recombination suggested that recombination might be lower in the heterogametic sex as a result of selection against crossing over between the sex chromosomes in this sex (Haldane, 1922; Huxley, 1928). Bell (1982) notes that this cannot be a complete explanation for achiasmate meiosis: White (1976) estimates that it has evolved some 8 times in the Mantodea, yet in each instance males were XO heterogametic, so crossing over between sex chromosomes could not have been possible in males anyway. Here we conclude that the explanations of Haldane and Huxley also cannot satisfactorily account for observed quantitative sex differences in chiasma frequency.

The only consistent sex difference observed was that in the Liliaceae there are more chiasmata formed in the embryo mother cells (female) than in the pollen mother cells. It is difficult to relate this observation to the various theories because the relevant plant population biology is unknown. For example, while among animals the variance in reproductive success tends to be higher in males than in females (Clutton-Brock, 1988), the only direct study on a plant gave ambiguous results. Meagher (1986) studied seeds collected from known female parents of Chamaelirium luteum, a dioecious lily, and estimated paternity using genetic markers; he found that variance in the number of mates was higher for males than females ($F_{58,68}$=7.7). However, in a study of established seedlings, from which both the mothers and the fathers were estimated, variance in the number of mates and the number of progeny was higher among females than among males ($F_{136,183}$=3.15 and $F_{136,183}$=4.19 respectively; Meagher and Thompson, 1987). Similarly, several diversity theories of recombination emphasize the importance of dispersal patterns (e.g. Williams, 1975; Bell,

1982; Tooby, 1982), and so one could derive predictions from any differential dispersal of genes transmitted through pollen and ovules. While one would expect that genes transmitted through pollen should be scattered further than those through ovules, since they have an extra round of dispersal, nevertheless established seedlings of C. luteum were found significantly closer to their father than to their mother (8.9 vs 10.1m; Meagher and Thompson, 1987).

Thus it is not clear how well any particular theory based on sex-specific costs and benefits can account for the trend in the Liliaceae. In any case, no such theory seems to account in any obvious way for the considerable variance in sexual dimorphism outside this family. Further, the very idea of sex-specific optima suggests that the magnitude of sexual dimorphism should be correlated with the opportunity for sex differences in selection. To test this idea we compared dioecious animals, plants, and hermaphroditic animals; the absence of a significant difference among these groups contradicts the hypothesis. Indeed, it is rather difficult to imagine how any selective differences might account for the large sex differences observed in some Platyhelminthes. Perhaps an alternative approach is required.

ARE SEX DIFFERENCES IN RECOMBINATION NEUTRAL?

To now we have assumed that selection on recombination determines a simple individual optimum and that sex differences in recombination are due to sex differences in this optimum. The repair theory of Bernstein et al. (1988) is a particularly clear example of this type of theory. However, it is also possible that selection determines only the population mean recombination rate (analogous to determining a 50:50 sex ratio) and that individual optima will depend on what others in the population are doing. In the present context, the optimum for males may depend on the females' rate of recombination, and vice versa. Theories of linkage disequilibrium decay fit this mold well.

Suppose there is linkage disequilibrium (l.d.) among the males of a population, but not the females. Then, since the sexes contribute equally to the next generation, recombination in females will have no effect on population l.d. On the other hand, if l.d. is equal in the two sexes, then the

mean amount of recombination in the two sexes will determine the rate of decay; sex differences per se will have no effect. Thus, if the function of recombination is to reduce l.d., then the potential for sex-differential optima of recombination is restricted to instances where there are sex differences in the amount or pattern of l.d. Furthermore, one can divide the l.d. in a population at time of reproduction into the fraction which was created in that generation, and the remainder, which is a holdover from all previous generations. Only the former can differ consistently between the sexes, due to sex-specific epistasis or selection: just as the sexes start each generation with equal gene frequencies (assuming a large zygote population), so they start with equal l.d. (This need not be so: one can imagine a meiotic system in the heterogametic sex in which recombinant chromosomes segregate with one type of sex chromosome and parental chromosomes with the other. Other mechanisms can be imagined for haplodiploid and monogenous species. However, we know of no example.) At equilibrium, when the l.d. created by selection equals that destroyed by recombination, the l.d. created in one generation between unlinked loci will be only one half the total l.d.; for more closely linked loci, this fraction will be correspondingly lower.

Thus, if recombination functions to reduce linkage disequilibrium, then there is unlikely to be strongly dimorphic selection pressures between the sexes. Indeed, Nei's (1969) simulation study of recombinational load using different fitness matrices for the two sexes found only very slight effects of sex differences in recombination on population mean fitness - too small, he suggests, to be selected. These very slight differences in optima may be swamped by other factors, such as differences in the mechanical cost of chiasmata or historical contingencies. For example, if there is selection on the population for increased recombination, then whichever sex has more additive genetic variance for rates of recombination may respond more, and thus the sexes may diverge over time. The absence of a consistent sex difference across species would not be mysterious, but expected.

In its strongest form, this theory suggests that sex differences in recombination are invisible to natural selection and that species will drift up and down lines of neutral equilibria representing isoclines of equal total recombination. This does not seem to be an accurate description of the

data, as the chiasma frequency of males and females is much more similar than this hypothesis would lead one to predict (Fig. 2).

A slightly modified theory suggests that sex differences are more-or-less neutral only in the region of the line of equality, affecting mean fitness only at some distance away. Such a situation may arise, for example, if the mechanical or physiological costs of crossing over increase with increasing numbers of cross-overs. To test this modified theory we take a different tack.

The neutral theory of sex differences predicts that experimental manipulations of recombination in one sex will result in selection for compensation by the other. Unfortunately, this prediction is difficult to assess at the moment: we are not aware that any such experiment has been done, and interpreting comparative relations in terms of tradeoffs is notoriously difficult, as recent experience with the cost of reproduction has demonstrated (Bell and Koufopanou, 1986). For example, the positive correlation of male and female chiasma frequencies across species (Fig. 1, Table 1) may seem to contradict the prediction of compensation, but actually is to be expected simply if the between-species variance in mean chiasma frequency is greater than the variance in sexual dimorphism. The most relevant comparisons are those which appear to be a 'natural experiment' - a seemingly randomized effect, if not actually manipulated. Here we consider three such situations: achiasmate species, haplodiploid species, and the sex chromosomes.

The comparison of chiasmate and achiasmate species is the least satisfactory of the three. On the one hand, the fact that achiasmy is only ever observed in one sex supports the prediction of compensation. (Christensen's (1961) study of hermaphroditic enchytraeid annelids is often cited as the one example of achiasmate meiosis in both sexes. Subsequent study indicated that spermatogenesis in these worms is in fact chiasmate (Christensen 1980).) On the other hand, there is no indication of compensation in the chiasma frequencies of the recombining sex (Fig. 1). However, this result may simply indicate that an achiasmate meiosis reflects selection for reduced recombination, which is also acting on the other sex.

Haplodiploidy seems a more promising natural experiment to test the hypothesis, for it is less likely that this genetic system has evolved and is maintained by selection for reduced recombination. Unfortunately, data is

available for only a single species (Aphytis mytilaspidis), and this exists in both sexual (haplodiploid) and asexual (automictic) forms (Rossler & DeBach 1973). It appears, then, that there has been selection for reduced recombination in this particular species, and so it is perhaps not surprising that there is no indication of compensation in the females' chiasma frequency (Fig. 1). Comparable data on more haplodiploid species would be of interest.

The final example is a comparison of chiasma frequencies of the sex chromosomes and the autosomes in the heterogametic sex. In female mice the sex chromosomes have more chiasmata for their length then the autosomes (Fig. 3), perhaps compensating for the much lower levels of recombination in the male. Alternative explanations for this observation seem possible, for the absence of recombination in males is not the only difference between the sex chromosomes and the autosomes. Perhaps most importantly, the X-chromosome is hemizygous in the male, resulting in lower mutation rates (Cavalli-Sforza and Bodmer, 1971; Miyata et al., 1987), greater sensitivity to founder events (Templeton, 1987), faster rates of evolution (Charlesworth et al., 1987), and perhaps altered rates of l.d. production. One possible test to distinguish these theories would be to look at achiasmate species: the theory of compensation predicts that in such species there should be no difference between sex chromosomes and autosomes in the homogametic sex; theories based on the hemizygous nature of the X-chromosome in the heterogametic sex predict differences as large as those in chiasmate species.

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APPENDIX

No. is the number used in Fig. 1; Gender system (h-hermaphrodite; d-dioecious, with sex chromosome system; amphibians from Sims et al. 1984 and Duellman & Trueb 1986); n is the haploid number of autosomes; Xta freq is total number of chiasmata formed on these bivalents; Diff indicates whether the author(s) claim there is a real sex difference (y-yes; n-no; those in parentheses are not based on a statistical test); Com. are numbered comments, listed at the bottom; Ref refers to numbered entries in the bibliography.

Taxon No.	Gender system	Ploidy	Autosomes n	Xta freq Male	Xta freq Female	Diff	Com	Ref.
CHIASMATE SPECIES								
Platyhelminthes: Trematoda								
1 Paradistomoides orientalis	h	2	14	32.3	32.3	n	1	10
Platyhelminthes: Turbellaria								
2 Notoplana igiliensis (Polycladida)	h	2	10	12.5	18.6	(y)		14
3 Dendrocoelum lacteum (Tricladida)	h	2	7	11.8	20.4	y	2	40
4 Gyratrix hermaphroditus (Neorhabdocoela)	h	2	3	5.2	4.5	-	3	27
Insecta: Orthoptera: Acrididae								
5 Eyprepocnemis plorans (Eyprepocnemidinae)	d-XO/XX	2	11	14.1	12.0	y	4	5
6 Melanoplus femur-rubrum (Melanoplinae)	d-XO/XX	2	11	13.5	14.0	n	4	8
7 Chorthippus brunneus (Gomphocerinae)	d-XO/XX	2	8	13.6	13.1	n	4	24
8 Chrysochraon dispar (Gomphocerinae)	d-XO/XX	2	8	12.6	12.1	y	4	12
9 Myrmeleotettix maculatus (Gomphocerinae)	d-XO/XX	2	8	14.4	13.2	y	4	20
10 Chortoicetes terminifera (Oedipodinae)	d-XO/XX	2	11	13.1	11.6	y	4	12
11 Parapleurus alliaceus (Oedipodinae)	d-XO/XX	2	11	12.3	12.9	y	4	12
12 Stethophyma grossum (Oedipodinae)	d-XO/XX	2	11	11.3	13.7	y	4	41
Chordata: Amphibia								
13 Rana esculenta (Anura)	d-XY/XX	2	13	25.2	45.7	-		15,51
14 Salamandra salamandra (Urodele)	d-??/??	2	12	24.0	36.8	-		29
15 Triturus alpestris (Urodele)	d-XY/XX	2	12	32.3	24.5	-	5	19,28
16 T. helveticus	d-XY/XX	2	12	22.0	25.0	(y)		49
17 T. cristatus	d-XY/XX	2	12	36.5	24.0	y		49
18 T. marmoratus	d-XY/XX	2	12	25.7	29.0	-		19,34

Chordata: Mammalia									
19	Sminthopsis crassicaudata (Marsupialia)	d-XY/XX	2	6	13.6	10.2	(y)		1
20	Mus musculus (Rodentia)	d-XY/XX	2	19	20.9	28.9	y		23
21	Macaca mulatta, nemestrina (Primates)	d-XY/XX	2	20	39.6	31.7	-		7,11,22
22	Papio papio (Primates)	d-XY/XX	2	20	41.5	39.6	-	6	7,22
Angiospermae: Dicotyledonae: Leguminosae									
23	Trigonella foenum graecum	h	4	16	21.3	21.1	(n)	7	44
24	Vicia faba	h	2	6	20.6	16.0	(y)		16
Angiospermae: Monocotyledonae									
Commelinaceae									
25	Rhoeo discolor	h	2	6	10.2	11.4	-		6
Gramineae									
26	Hordeum vulgare	h	2	7	13.9	13.7	(n)		2
27	Secale cereale	h	2	7	10.7	10.6	n		9
Liliaceae									
28	Allium cepa	h	2	8	22.4	17.9	y		17
29	A. consanguineum	h	2	8	21.9	17.5	y		17
30	A. flavum	h	2	8	14.9	18.8	(y)		46
31	A. macranthum	h	4	14	42.3	58.7	(y)		46
32	A. nigrum	h	2	8	21.9	16.9	y		17
33	A. pallens	h	2	8	15.0	19.4	(y)		46
34	A. paniculatum	h	2	8	14.6	16.0	(y)		46
35	A. ursinum	h	2	7	13.8	14.1	(y)		46
36	Endymion nonscriptus	h	2	8	17.7	18.2	n	8	52
37	Fritillaria meleagris	h	2	12	24.8	37.8	(y)		13
38	Lilium hansonii	h	2	12	40.0	49.0	-		4
39	L. henryi	h	2	12	41.2	44.4	(y)		13
40	L. longiflorum	h	2	12	27.3	31.5	(y)		13
41	L. martagon	h	2	12	36.3	41.0	(y)		13
42	L. pardalinum	h	2	12	31.2	36.0	(y)		13
43	L. regale	h	2	12	41.8	45.0	(y)		4,13
44	L. sargentiae	h	2	12	31.2	42.0	(y)		13
45	L. speciosum	h	2	12	26.4	33.9	-		3
46	Tulbaghia acutiloba	h	2	6	14.4	15.8	(y)		47
47	T. leucantha	h	2	6	12.4	15.5	(y)		47
48	T. pulchella	h	2	6	12.2	13.7	(y)		47
49	T. violacea	h	2	6	11.0	14.3	(y)		47

Orchidaceae								
50 Cypripedium cordigerum	h	2	10	16.4	19.7	(y)		32
51 Epipactis consimilis	h	2	20	25.8	27.1	(y)		30
52 E. latifolia	h	2	20	30.7	29.1	(y)		30
53 Listera ovata	h	2	17	26.9	30.3	y		48
54 Neottia listeroides	h	4	20	29.3	31.1	(y)	9	31
ACHIASMATE SPECIES								
Platyhelminthes: Turbellaria								
55 Mesostoma ehrenbergii (Neorhabdocoela)	h	2	5	3.0	0.0			37,38
Insecta								
56 Thericles whitei (Orthoptera)	d-XO/XX	2	8	0.0	8.2		10	50
57 Drosophila melanogaster (Diptera)	d-XY/XX	2	4	0.0	5.0		10	43
58 Allogamus auricollis (Trichoptera)	d-ZW/ZZ	2	30	60.0	0.0		10	25
59 Glyphotaelius pellucidus (Trichoptera)	d-ZW/ZZ	2	30	35.0	0.0		10	26
60 Antheraea assamensis, compta (Lepidoptera)	d-ZW/ZZ	2	15	20.0	0.0		10	18
61 Bombyx mori (Lepidoptera)	d-ZW/ZZ	2	28	58.0	0.0		10	21
62 Byblia ilithyia (Lepidoptera)	d-ZW/ZZ	2	17	17.4	0.0		10	33
63 Ephestia kuehniella (Lepidoptera)	d-ZW/ZZ	2	30	30.0	0.0		10	45
64 Philosamia ricini (Lepidoptera)	d-ZW/ZZ	2	14	22.0	0.0		10	39
Angiospermae: Monocotyledonae: Liliaceae								
65 Fritillaria amabilis	h	2	11	0.0	27.0			35,36
66 F. japonica	h	2	11	0.0	35.2			35,36
67 F. koidzumai	h	2	12	0.0	51.0			35,36
HAPLODIPLOID SPECIES								
Insecta: Hymenoptera								
68 Aphytis mytilaspidis	d	2	5	0.0	5.5			42

Comments

1-Female chiasma frequency given only as "not significantly different from the male".

2-Male chiasma frequency at metaphase I; female value at diplotene.

3-Chiasma frequencies inferred from verbal descriptions.

4-Sex bivalent in females cannot be distinguished from at least some autosomes; assumed it had the mean chiasma frequency.

5-Male chiasma frequency for subspecies cyreni; female value for ssp. apuanus.

6-Not clear if the male chiasma frequency included the sex bivalent; subtracted 0.5 to get the autosomal chiasma frequency.

7-Induced polyploid.

8-Assumed n=8 (Ved Brat 1966 does the same).

9-Some male meiosis is abnormal, leading to unbalanced pollen.

10-Haploid numbers and chiasma frequencies for dioecious achiasmate species include the sex bivalent.

CHAPTER FIVE

Comparative methods using phylogenetically independent contrasts†

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INTRODUCTION

As the data base of biology grows at its ever increasing pace, comparisons among species are becoming an increasingly popular means of testing evolutionary and ecological theory. Among the more common analyses are tests of association between two variables across some defined set of species; the variables of interest may be properties of individuals (e.g. testis size, Harcourt et al. 1981), populations (e.g. genetic polymorphism, Nevo et al. 1984), or of the whole species (e.g. number of pests, Strong et al. 1984). Concurrent with this trend has been a growing appreciation of the statistical properties of such comparisons, in particular the 'problem of phylogeny', and a widening array of statistical methods from which a comparative biologist may choose (e.g. Harvey & Mace 1982; Ridley 1983; Clutton-Brock & Harvey 1984; Felsenstein 1985; Bell in press; Grafen in press; see review by Pagel & Harvey 1988a). In this paper I try to put some of the issues into perspective and to expand upon one particularly promising approach to working with comparative data. I first discuss in detail the consequences of phylogenetic similarity - the common observation that closely related species are more similar than distantly related species - for the inferences one may draw from species-level correlations. These considerations lead to the notion of dividing a phylogeny into a series of independent replicate comparisons; these 'contrasts' may then be used in tests of association which are more discriminating - and thus often more interesting - than species-level analyses. They may also be used in conjunction with information on times of divergence in the evolutionary tree to address a number of explicitly historical topics, such as the relationship between speciation and phenotypic change, the suitability of random walk models of evolution, and the notion of phylogenetic inertia. I shall illustrate all analyses with the data of Sessions & Larson (1987) on DNA content and differentiation rate in plethodontid salamanders.

PHYLOGENETIC SIMILARITY

If one has an hypothesis that X plays some role in determining Y, then one obvious test is to look for a correlation between X and Y, using each

species for which data are available as an independent data point. However, this practise has recently been the target of some extensive criticism (e.g. Harvey & Mace 1982; Ridley 1983; Felsenstein 1985; Grafen 1989), though accounts differ in identifying the exact source of the problem. Here I make use of results from the computer simulations of Raup & Gould (1974). These authors generated a phylogenetic tree from a single ancestral species by an iterative process of random speciation and extinction, until there were 200 descendants. Phenotypic evolution of 10 hypothetical characters was then modelled by 10 independent random walks: each character started from an ancestral value of zero and evolved by randomly increasing or decreasing one unit (or staying the same) at each branch point. Raup & Gould then calculated the correlation coefficient for all 45 pairwise combinations of the 10 variables. As can be seen in figure 1, the frequency distribution of their correlation coefficients is much wider than that expected from 200 pairs of normally distributed random variables - indeed, fully 45% of their correlation coefficients are significant by the conventional test, despite the fact that the characters evolved by independent random walks. This difference between the observed distribution of correlation coefficients and the conventional theoretical distribution is due to phylogenetic similarity: because descendant phenotypes are modifications of ancestral phenotypes, closely related species are likely to be more similar than distantly related species. Significant correlations arise because species with similar X-values are likely to be closely related and thus also likely to have similar Y-values, independent of any causal mechanism relating changes in X to changes in Y. Note that it does not really matter <u>why</u> closely related species are more similar than distantly related species - be it because of adaptation to common environments, phylogenetic inertia, developmental constraints, or whatever - but as long as they are, then X and Y will tend to be correlated. These simulations demonstrate that significant interspecific associations between two variables may be due not only to causal mechanisms, simple or complex, but also to the rather common (and perhaps boring) tendency for closely related species to be more similar than distantly related species. A statistical test which could distinguish between these possibilities would be very useful.

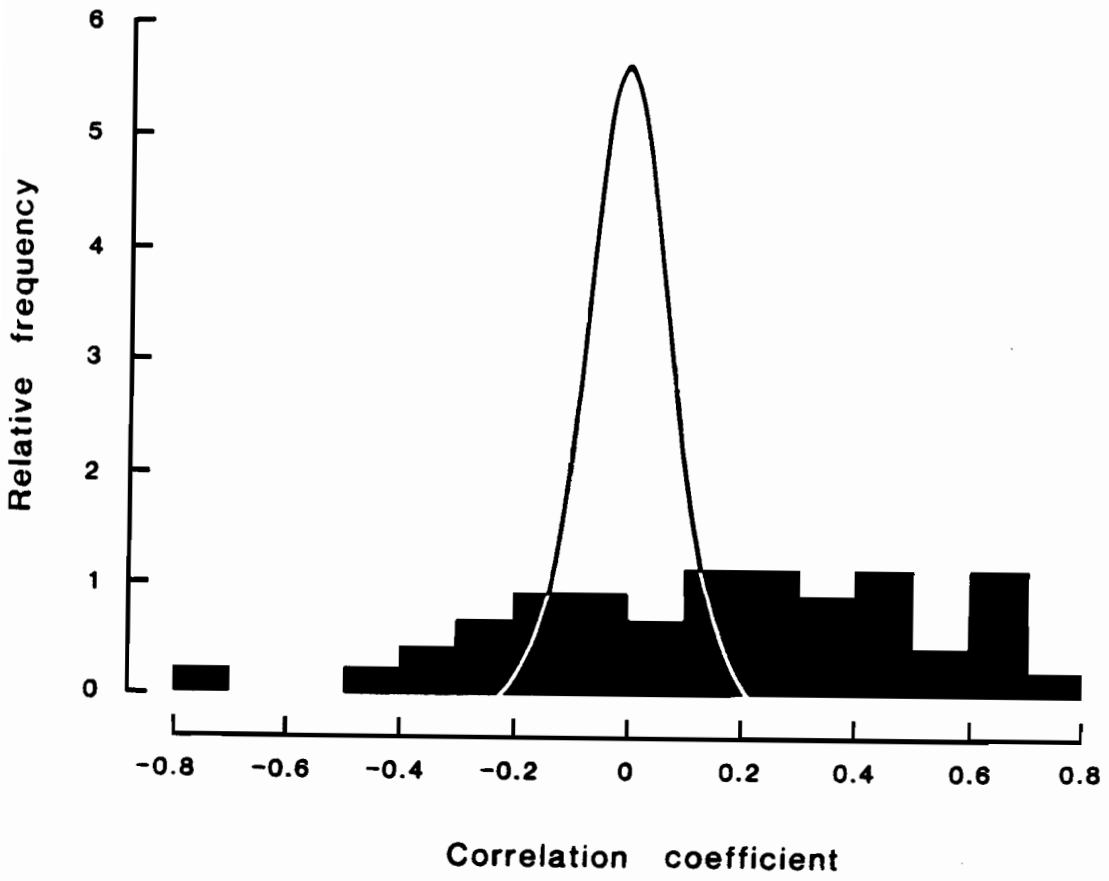

Figure 1. The problem of phylogenetic similarity. The histogram is a frequency distribution of correlation coefficients from the computer simulations of Raup & Gould (1974: Table 2). Phenotypic evolution was modelled as independent random walks along a simulated phylogenetic tree with 200 descendant species. Forty-five pairwise correlation coefficients are shown, as well as the expected distribution of correlation coefficients for n=200 pairs of normally distributed random variables (normal distribution with mean=0 and variance=1/198; Sokal & Rohlf 1981:583). The correlation coefficients are from only one tree and are all pairwise combinations of only 10 variables; nevertheless, the sizeable difference between null models is clear. In particular, note that the distribution of simulated correlation coefficients is much wider than the theoretical curve, resulting in many statistically significant values under the conventional tests of association, despite the fact that the characters evolved by independent random walks.

Before describing such a test, a few further comments on species-level analyses seem appropriate. Consider Raup & Gould's 200 simulated descendant species as an order of mammals with parametric correlation coefficient between X and Y equal to ρ. If one were to sample randomly from this population and calculate the correlation coefficient and associated probability level for this sample, these statistics would have many of the conventional interpretations: the sample correlation coefficient r would still be a good estimate of ρ and the p-value would still give the probability that the parametric ρ has sign opposite to that of the sample r. For some purposes these statistics may be all that is required, with rather little depending on whether an association is causal or due to phylogenetic similarity. On the other hand, the simulations of Raup & Gould indicate that these statistics may be a very poor indicator of the correlation in another order of mammals, even if the same biological processes were occuring - if associations are completely due to phylogenetic similarity then a significant positive association in one order is just as likely to be negative in another. Certainly, inferring causality from a correlation (i.e. extrapolating to the outcome of manipulative experiments) will be even more tenuous than usual if there is a good chance that the correlation is due to phylogenetic similarity. To conclude, species-level analyses are not 'wrong', and may in fact reveal much of interest; however, they do permit fewer inferences than other more powerful techniques.

PHYLOGENETICALLY INDEPENDENT CONTRASTS AND TESTS OF ASSOCIATION

There are numerous comparative methods in the literature which attempt to deal in some way with the problem of phylogenetic similarity (reviewed in Pagel & Harvey 1988a); briefly, these may be divided into three groups. First, one can compare across higher taxa, such as genera or families, instead of species. For example, Harvey & Clutton-Brock (1985) use subfamilies in their analysis of primate life history data. Second, one can control for taxonomic affiliation directly by including it as a categorical variable in a multiple regression model. The methods used by Stearns (1983) and Wootton (1987) in their analyses of mammalian life history data are elaborations on this theme. Computer simulations by

Grafen (1989) suggest that while using either of these methods does reduce the likelihood that a significant association is due to phylogenetic similarity, neither one completely excludes this alternative explanation. Finally, one can divide the data set into a series of phylogenetically independent replicate comparisons, each of which contributes one degree of freedom to the subsequent test of association. It is this latter approach which I will pursue here.

Consider again the simulations of Raup & Gould (1974). In any one particular simulation the correlation between two variables may be highly significant under the conventional test. However, in repeated simulations the expected correlation is zero - sometimes 'significantly' positive, sometimes negative, but with a long-term expected mean of zero. In theory, then, one could test for an association between two variables in an order of mammals by evolving the order repeatedly and seeing if there is a tendency for positive or negative correlation coefficients to predominate. In practice, one may proceed in a completely analogous manner: divide the order into independent replicate groups containing at least two species and then see if there is a tendency for positive or negative trends within groups to predominate. This is the method of phylogenetically independent contrasts.

Replicating comparisons within a taxon is not a particularly new idea, though it has a far from consistent history in the comparative literature. For example, to test for an association between rates of recombination and breeding system, Brown (1961) compares chiasma frequencies within six pairs of closely related species or subspecies in the genus Gilia (Polemoniaceae), where each pair consists of a selfer and an outcrosser. More recently, Felsenstein (1985:13) cites essentially the same suggestion: one should compare pairs of nearest relatives, "two seals, two whales, two bats, two deer, etc.". Read (1987) takes a slightly different approach in his test of association between parasite load and plumage coloration in passerines, calculating Spearman rank correlations separately for each genus in his data set and then testing for mean different from zero. Here I extend these methods to the problem of extracting independent contrasts from a known phylogeny; Felsenstein (1988:457) independently describes much the same method. First, though, I introduce the example data set.

Simple biochemical considerations suggest that if most of the interspecific variation in genome size is in the non-coding fraction then the more DNA there is, the longer will be the cell cycle and the slower the rate of development. These ideas suggest that there will be a negative correlation between DNA content and developmental rates across species; to test this prediction, Sessions & Larson (1987) collected data on the C-value (haploid DNA content) and the differentiation rate of regenerating limbs for 27 species of plethodontid salamanders. These data are reproduced in figure 2. (See original paper for more details on theories and variables.) There is indeed a significant negative correlation between C-value and differentiation rate (Fig. 3a) and the parametric correlation coefficient for the Plethodontidae seems to be quite low (assuming this is a random sample, 95% confidence limits are -0.69 and -0.93). Thus the causal hypothesis gains some measure of support. However, this test does not reject the null hypothesis that C-value and differentiation rate have evolved independently of one another: as demonstrated above, such associations are not improbable if there is considerable phylogenetic similarity. This alternative explanation is supported by an analysis of variance, which indicates very highly significant differences between the two subfamilies in the data set in both C-value and differentiation rate ($F_{1,25}$ = 21.9 and 45.3 respectively, both $p<0.001$). Estimates of the variance components indicate that over 75% of the total variance in both C-value and differentiation rate lies between subfamilies; these considerable differences are also evident from the scatterplot (Fig. 3a). Thus closely related species are much more likely to be similar than distantly related species - is this fact alone responsible for the observed correlation?

To further test the causal hypothesis I divide the data set into a series of replicate contrasts. A contrast is simply a group of two or more species; it can be represented as a path joining the member species through the phylogeny. I suggest that in tests of association one only use contrasts whose paths do not meet at any point; contrasts which satisfy this criterion are "phylogenetically independent". Note that variances and covariances of phenotypic characters within contrasts, which are assumed to depend only on evolutionary events occuring since the last common ancestor, will thereby be independent of variances and covariances in other contrasts. The

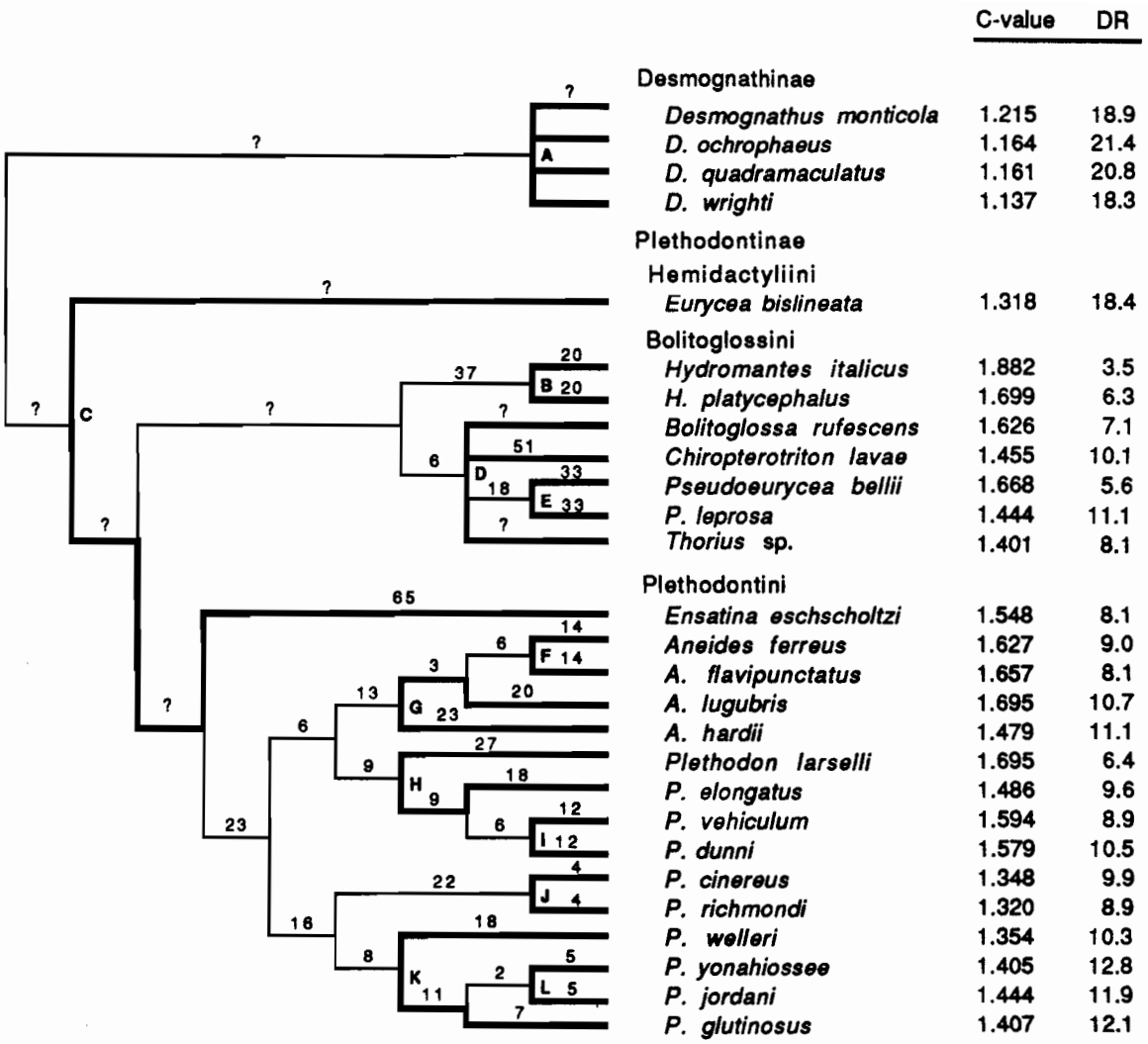

Figure 2. The data of Sessions & Larson (1987) on C-value (haploid DNA content in pg, log-transformed) and differentiation rate of regenerating hind limbs (units are (developmental stages per day) x 100) in plethodontid salamanders. To the left are phylogenetic relationships, inferred from morphological and biochemical data, which are used in identifying independent contrasts. Contrasts are groups of at least two species and may be represented as a path joining the member species through the phylogeny; two or more contrasts are phylogenetically independent if their paths do not meet or fall along the same vertical line. For 27 species there can be a maximum of 13 phylogenetically independent contrasts, but since the phylogeny contains multiple-branching nodes the maximum here is only 12 (A-L). There are numerous ways of dividing the phylogeny into fewer contrasts, not shown. The numbers in the phylogeny, also from Sessions & Larson (1987), are times of divergence estimated from albumin immunological distances and Nei's electrophoretic distances on the assumption that these increase linearly with time (i.e. are good 'molecular clocks').

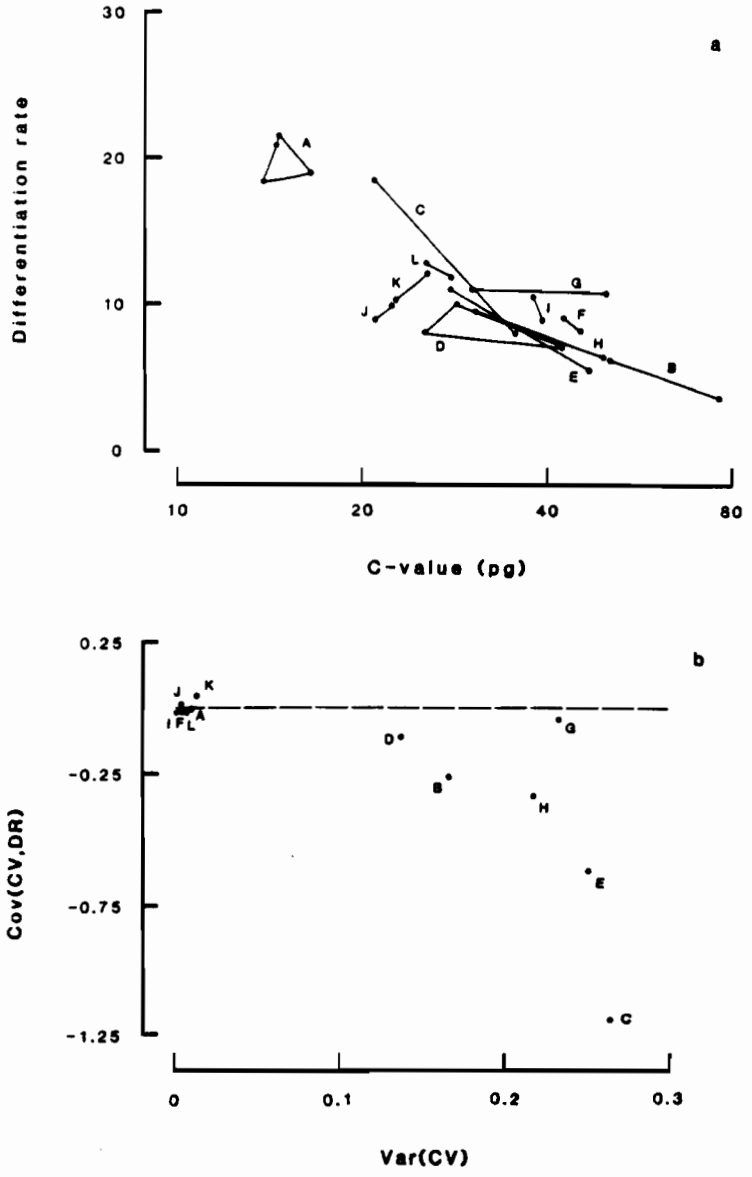

Figure 3. Two views of the relationship between differentiation rate and C-value. (a) The standard species scatterplot shows a very highly significant negative correlation between species means ($r=-0.85$, $n=27$, $p<0.0001$). Lines connect members of phylogenetically independent contrasts, lettered as in Fig. 2. Note that the subfamily Desmognathinae (contrast A) is quite separate from the subfamily Plethodontinae (all the rest), indicating strong phylogenetic similarity. (b) Each contrast is reduced to a single point in this graph of the covariance of C-value and differentiation rate as a function of the variance in C-value. Note that the slope of the line connecting each point to the origin is the least-squares slope of the relation within contrasts. Data from Table 1.

same is not true of random, even if non-overlapping, groups of species: the difference between a mouse and a macaque is not independent of the difference between a rat and a chimp. Figure 2 shows the data set of Sessions & Larson divided into 12 phylogenetically independent contrasts and Table 1 shows the variances and covariances of C-value and differentiation rate calculated for each contrast. A useful graphical method of representing the contrasts is to plot the covariance of C-value and differentiation rate as a function of the variance in C-value (Fig. 3b): the slope of the line connecting each data point to the origin is the least-squares slope of differentiation rate on C-value within that contrast [b=Cov(X,Y)/Var(X)].

To test for an association between C-value and differentiation rate one can simply apply a sign test to the covariance estimates. The null hypothesis is that the probability of a covariance being negative equals the probability that it is positive. Here 10 of 12 covariances are negative (p=0.039, 2-tailed), indicating a significant tendency for increases in C-value to be associated with decreases in differentiation rate, and vice versa, and rejecting the hypothesis that the species-level correlation is wholly due to phylogenetic similarity in these two characters. These results corroborate those of Sessions & Larson.

As the force of these conclusions derives from the criterion of phylogenetic independence defined above, several comments about it seem appropriate. First, there are many different ways of dividing a phylogeny into independent contrasts and often there will be a tradeoff between increasing the number of contrasts and increasing the proportion of variance which is 'within' contrasts (and thus being tested), as opposed to 'between' contrasts (and thus not being tested). It would seem appropriate to increase both statistics, but there is no obviously 'best' compromise. In the above analysis I maximized the number of contrasts, with the result that only 26% of the variation in C-value and 27% of the variation in differentiation rate lies within contrasts. In general for n species and a dichotomously branching phylogeny there are a maximum of n/2 phylogenetically independent contrasts (rounded down to the nearest integer), though fewer if the phylogeny contains multiple-branching nodes, as in the above example.

Table 1. The variances of C-value (CV) and differentiation rate (DR) and their covariance calculated separately for each of the phylogenetically independent contrasts indicated in Fig. 2. $Cov(X,Y) = [\Sigma XY - \Sigma X \Sigma Y/n]/(n-1)$; n = number of species.

Contrast	n	Var(CV)	Var(DR)	Cov(CV,DR)
A	4	0.00108	2.203	-0.0032
B	2	0.01674	3.920	-0.2562
C	2	0.02645	53.045	-1.1845
D	3	0.01380	2.333	-0.1050
E	2	0.02509	15.125	-0.6160
F	2	0.00045	0.405	-0.0135
G	2	0.02333	0.080	-0.0432
H	2	0.02184	5.120	-0.3344
I	2	0.00011	1.280	-0.0120
J	2	0.00039	0.500	0.0140
K	2	0.00140	1.620	0.0477
L	2	0.00076	0.405	-0.0176

However the phylogeny is divided, recognizing independent contrasts by the above criterion will allow one to use more of the data than if one restricted contrasts to pairs of nearest relatives or to just one level of the Linnean hierarchy. Of course, this is only an improvement if the extra information used is correct: the effect of errors in the phylogeny will presumably be similar to taking random groups of species as independent contrasts, which in the limit is no improvement on the standard species-level analysis. Thus, when in doubt about the phylogeny, the conservative solution is to use multiple modes.

On the other hand, the criterion of independent contrasts defined here is quite conservative in that values for a species are used only once; information on phylogenetic relationships among the contrasts is not used. As we have seen, this results in a maximum of n/2 degrees of freedom in the subsequent test of association. Is there an alternative? Felsenstein (1985) and Grafen (1989) make suggestions about how to combine data from different contrasts to construct yet more contrasts, thus deriving one degree of freedom for each higher node in the phylogeny (df=n-1 for a dichotomously branching phylogeny). However, these methods rely on particular assumptions about how information from different contrasts is to be combined. Felsenstein's (1985) method, one of those actually used by Sessions and Larson, requires information on times of divergence for each node and the assumption that characters evolve by Brownian motion; Grafen (1989) suggests an even more complicated method which requires that a series of arbitrarily assigned branch lengths be correct. While these methods may well be of some use, the method described here is more economical of assumptions and should be useful for this reason.

A good phylogeny does not exist for many taxa and comparative biologists studying these groups will have to extract contrasts from the Linnean classification. How might this be done? An obvious extrapolation of the above method involves using a variety of taxonomic levels, as follows: count each genus with two or more species as a contrast, remove them from the data set, count the tribes with two or more remaining genera as contrasts, remove them from the data set, count subfamilies with two or more remaining tribes as contrasts, remove them, and so on up the Linnean hierarchy. However, it should be recognized that this approach depends on each taxon being strictly monophyletic, at least with respect to

the species in the data set. For better or worse, monophyly is not always a criterion in constructing higher taxa and so many Linnean taxa are not monophyletic. For example, the genus Plethodon in the salamander data set is paraphyletic (i.e. species on the same branch have been put in another genus, Aneides - see Fig. 2), with the consequence that a contrast including the whole genus would not be independent of a contrast including both Ensatina and Aneides, two other genera in the tribe Plethodontini (Fig. 2). Thus, if contrasts are to be extracted from a Linnean classification and the taxa as represented in the data set are thought to be paraphyletic, then the conservative method is to use only one level of the Linnean hierarchy in defining contrasts. Five contrasts at the generic level or three at the tribal level can be identified in the salamander data set.

Tests of association between two qualitative variables may also be done using phylogenetically independent contrasts. Such variables include modes of sex determination, ovi- vs viviparity, and the sort of nucleotide found at a particular locus. Here I consider the simplest case of two variables with two states each and to do so have transformed the C-value and differentiation rate data into qualitative variables (Fig. 4). The exact same contrasts as used for the continuous data could be used, but there is a problem: to be useful in a test of association, a contrast must contain both states of both characters and only two of the original contrasts meet this additional criterion (C and E). Thus, unless the data set is very large, one will probably want to divide the data set in such a way that all contrasts contain both states of both characters. Unfortunately the gain is slight in this data set, as there are a maximum of only three such contrasts (Fig. 4); even though the association in all three is in the same direction - large DNA with slow differentiation and vice versa - it is impossible to get a statistically significant result from a sign test. Thus the method of independent contrasts can be easily extended to tests of association between qualitative characters, but in general one will need a much larger data set (or more detailed phylogeny) in order to get the same number of useful contrasts as for continuous variables. This approach provides an alternative to the methods of Ridley (1983, 1986; see Pagel & Harvey 1988a), which rely on the assumption that cladistic techniques such as outgroup comparison can accurately identify ancestral states. The test described here

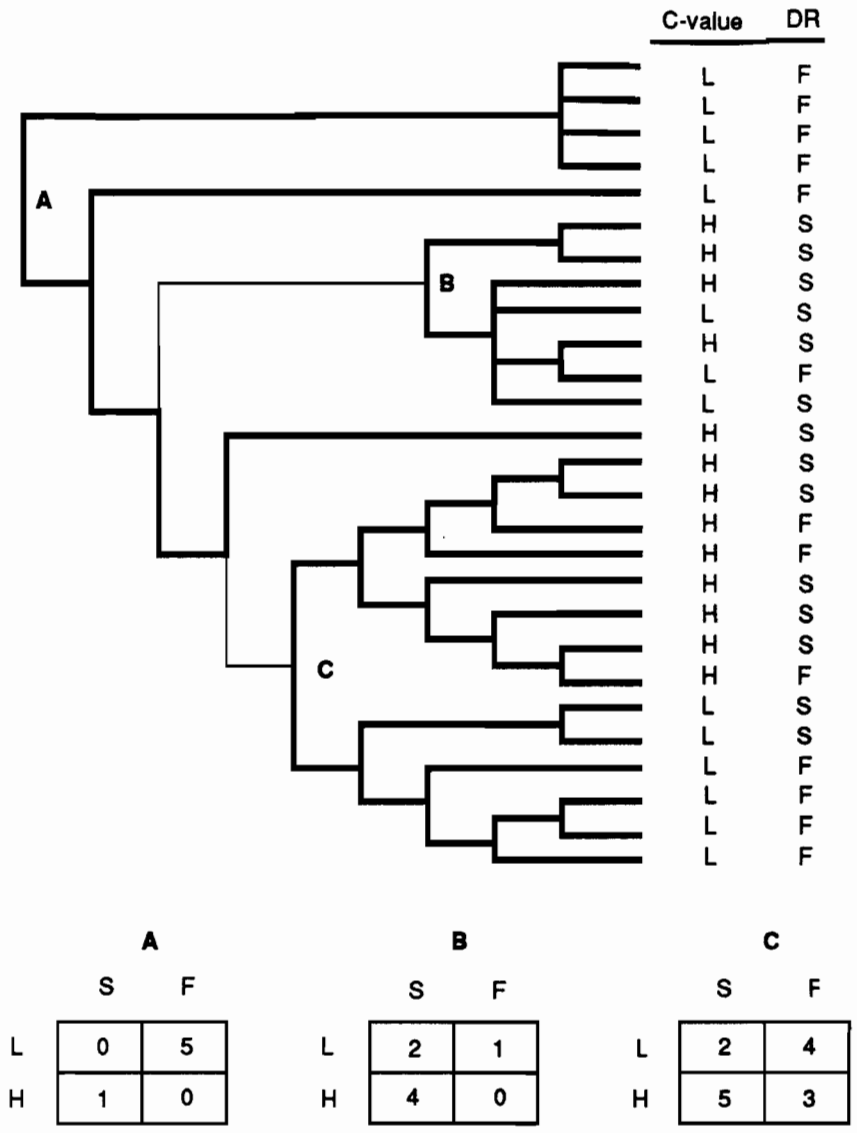

Figure 4. Test of association between two qualitative variables. Above. The data of Fig. 2 have been transformed into qualitative variables by classifying C-values as higher or lower than the median (H, L) and differentiation rates as faster or slower than the median (F, S). The phylogeny is unchanged. Phylogenetically independent contrasts are again identified, with the proviso that each one contains both values for both variables. There are a maximum of three such contrasts in the above tree; one possible set of three, with approximately equal numbers of species in each, is identified above (A-C). Below. Each contrast is represented as a 2 x 2 contingency table of species values. Note that the direction of association in all three contrasts is the same.

makes no assumptions about parsimony in evolution or the character states of ancestral species.

PHYLOGENETICALLY INDEPENDENT CONTRASTS AND EXPLICITLY HISTORICAL ANALYSES

One advantage of the above test of association is that it does not require data on the ages of each contrast; however, if such information is available then one can address a number of interesting historical questions. The most likely sort of data are molecular distances between extant species, which are being measured with increasing frequency for use in constructing phylogenies. The various uses of these data in aiding our understanding of phenotypic evolution has not, to my knowledge, been much considered, and is the topic of this section. I present a series of simple and emphatically exploratory analyses; again, all are illustrated with the salamander data, though, as will become obvious, significantly larger data sets will often be desirable. The branch lengths indicated in figure 2 are times of divergence (in millions of years) as estimated by a combination of albumin immunological distances and Nei's electrophoretic distances. These values may be regarded as a composite index of molecular divergence; they are available for nine independent pairwise contrasts with estimated times of divergence ranging from 4 to 33 million years ago (Table 2).

Phenotypic and molecular divergence. One obvious point of departure is the relationship between molecular and phenotypic divergence (Fig. 5 a, b). In these data it appears that differences in both C-value and differentiation rate are highly correlated with molecular divergence; indeed, the observed correlation coefficients (0.86 and 0.74 respectively) are about as high as those between the immunological and electrophoretic distances themselves (r=0.70 - 0.88; Maxson & Maxson 1979). Thus, if molecular divergence is a good measure of the age of a contrast then so is the divergence of C-value and differentiation rate, at least over the range considered here. The second point of interest is the intercept of regression lines fitted through the scatterplots. If speciation (cladogenesis) is typically associated with

Table 2. Phenotypic and molecular divergence in nine pairwise contrasts. Molecular divergences are the sum of branch lengths from figure 2 and those for C-value and differentiation rate are the absolute values of the difference between the two species.

Contrast	Divergence		
	Molecular	C-value	DR
B	20	0.183	2.8
E	33	0.224	5.5
F	14	0.030	0.9
G	23	0.216	0.4
H	27	0.209	3.2
I	12	0.015	1.6
J	4	0.028	1.0
K	18	0.053	1.8
L	5	0.039	0.9

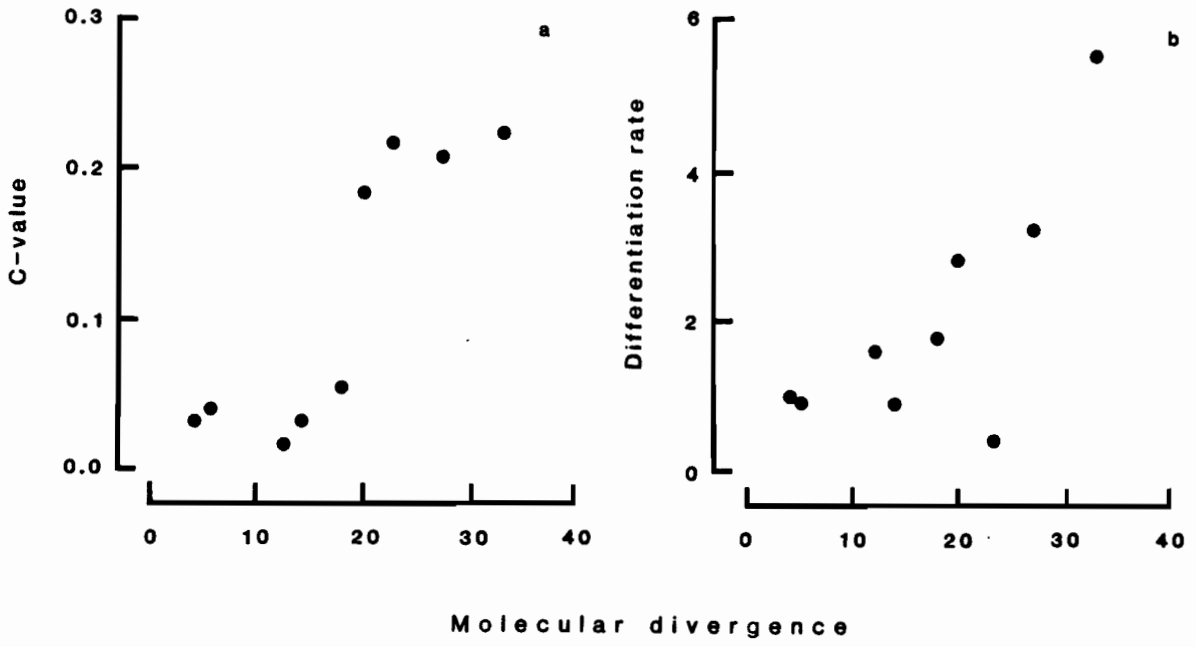

Figure 5. Phenotypic and molecular divergence in nine phylogenetically independent contrasts. Arithmetic plots show strong positive correlations between molecular divergence and differences in both C-value (a) and differentiation rate (b). Note that both functions increase approximately linearly and that both intercepts are not significantly different from zero, the latter suggesting that there are no 'punctuations' in C-value or differentiation rate associated with speciation.

particularly rapid phenotypic evolution, as suggested by some models of evolution (e.g. Gould & Eldredge 1977), then the regression of phenotypic divergence on time should have a positive intercept. However, both intercepts are very close to the origin (a_{CV} = -0.03 and a_{DR} = -0.12), suggesting that no very large punctuation in either C-value or differentiation rate typically occurs at time of speciation (or, at least no larger a punctuation than may occur in molecular divergence).

Random walk models of evolution. Further analysis is suggested by considering various null models of evolutionary change. I have already referred to one such model, the random walks of Raup & Gould (1974; see also Raup 1977). In this model changes during a time period of unit length have zero mean and constant variance and changes in successive time intervals are independent; consequently it predicts that phenotypic variances - not differences - should increase linearly with time. If the model is altered such that changes in successive time intervals are positively correlated, as might be expected under persistent directional selection, then variances should increase faster than linearly with time; on the other hand, a negative correlation between successive changes would predict that variances should go up more slowly than linearly with time. Thus, if we write

$$\text{Phenotypic variance} = a(\text{Time})^b \quad ,$$

then the exponent b is a measure of the autocorrelation of changes in successive time intervals, with b=1 when r=0. A log-log plot of the data, using the molecular divergence estimates of time, is shown in figure 6a. Unfortunately the data are too few to give precise estimates: neither the individual slopes nor the common slope are significantly different from the random walk prediction of 1, but the confidence limits are wide and do not exclude slopes of 1/2 or 2 either. Similar analyses on much larger data sets would be of interest.

[It should be noted that this analysis is only approximate, as variances based on only two values, even log-transformed, are unlikely to be normally distributed. Furthermore, the least-squares slope calculated will

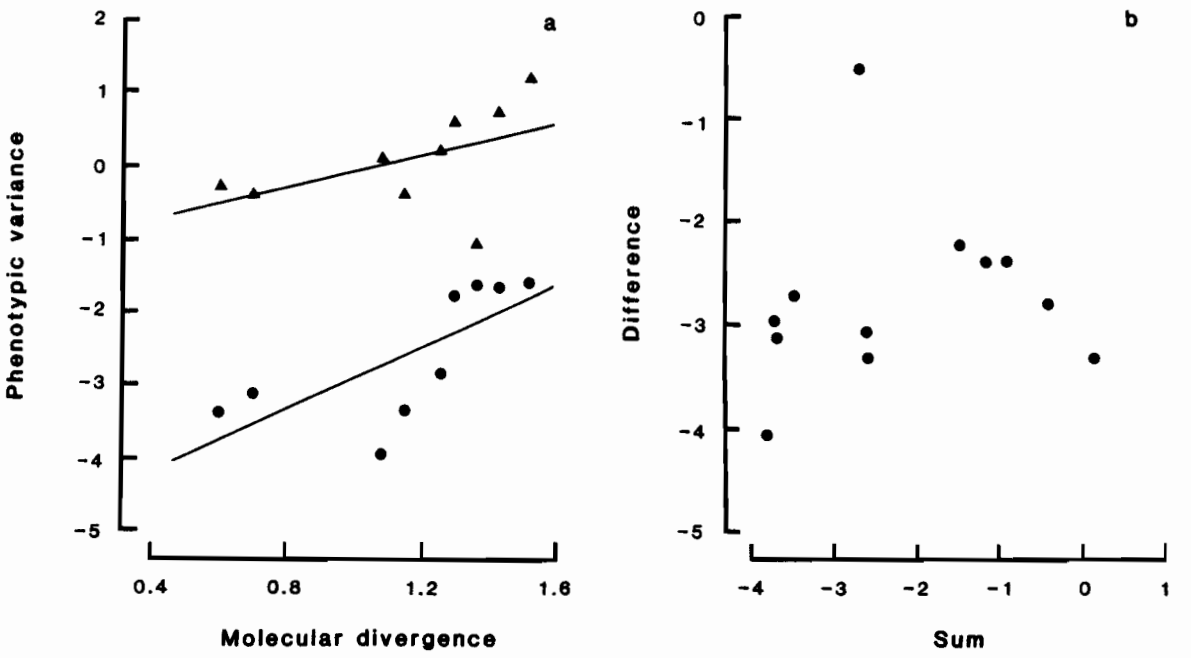

Figure 6. Testing null models of evolution. The assumptions of the molecular clock and random walk model of phenotypic evolution together predict that phenotypic variance should increase linearly with molecular divergence. (a) Log-log plot of phenotypic variance as a function of molecular divergence for C-value (●) and differentiation rate (▲) in nine phylogenetically independent contrasts. Neither the individual slopes ($b_{CV} = 2.1 \pm 1.79$ (95% C.L.) and $b_{DR} = 1.1 \pm 1.72$) nor the common slope ($b_C = 1.6 \pm 1.12$) are significantly different from the predicted slope of 1. Data from Tables 1 and 2. (b) In the absence of molecular comparisons, or other data on times of divergence, one can test the null hypothesis that the variance in the log-transformed estimates of variance are equal for two characters by plotting the difference of the estimates [logVar(CV) - logVar(DR)] as a function of their sum [logVar(CV) + logVar(DR)] for 12 phylogenetically independent comparisons; a positive correlation would indicate that $\sigma^2_{logVar(CV)} > \sigma^2_{logVar(DR)}$ and a negative correlation the opposite. In fact, there is no significant correlation ($r=0.220$, $0.5>p>0.4$).

tend to underestimate the true slope if there is a less than perfect correlation between molecular divergence and time. Corrections for this bias are possible, but require information on the relative error variances associated with X and Y (Pagel & Harvey 1988b). Finally, the interpretations assume that rates of evolution have been constant through time: conceivably, an alternative explanation for a slope greater than 1 is that evolution, though remaining a random walk, has gradually slowed down over the time period being studied.]

A further test of the random walk model is to check whether rates of evolution have been homogeneous in different contrasts. Variances within each contrast may be standardized by dividing by the estimated age (molecular divergence) of the contrast and then compared using Bartlett's test (see Snedecor & Cochran 1980:252 and tables in Pearson & Hartley 1966). Unfortunately, estimates of variance are very imprecise when based on only two values, and so this test has rather low power: not only are there no significant differences between the standardized variances for either C-value or differentiation rate, but there are also no detectable differences between the unscaled variances (for which we know there are real differences, since they are correlated with molecular divergence - Fig. 6a).

One conclusion that may be drawn from these results is that the simple tests of the random walk described here, using independent contrasts, are rather weak and will generally require many more than nine contrasts. One possible alternative approach would be to use the molecular data to calculate the complete series of contrasts defined by Felsenstein (1985). Such analyses are more complex, for one has to recalculate the contrasts for each value of the exponent b under test, but they do use more of the available information and thus should provide more precise estimates. Unfortunately, using the 18 such contrasts extractable from this data set (see Sessions & Larson 1987) one still cannot distinguish between exponents b=1 and b=2, nor detect significant heterogeneity in the unscaled variances of either C-value or differentiation rate. Thus, it seems the only solution is to increase the size of the data set, if possible also increasing the range of contrast ages under study.

These tests of the random walk are obviously restricted to instances where one has molecular data or some other measure of times of

divergence; can similar questions be addressed in the absence of such information? I suggest that while one may not be able to test a specific evolutionary model, such as the random walk, nevertheless one can test whether two phenotypic characters follow the same evolutionary model (whatever that may be). For example, one might have a theory that naturally and sexually selected characters should have different patterns of evolutionary change, or that characters closely related to fitness should differ from more neutral characters. One approach would be to compare the partitioning of variation at different taxonomic levels for the two characters. Unfortunately, formulae for confidence limits of variance components in unbalanced data sets are not known (Sokal & Rohlf 1981). Maximum likelihood methods of estimating variance components would seem to offer a possible solution, and as such would be worth further study; however, these are beyond the scope of this paper. Instead, I present an alternative method using independent contrasts, as follows. I suggested above that the slope of the regression of log-variance on log-time across contrasts estimates the exponent in the relationship Variance = $a(Time)^b$. If two phenotypic characters have the same exponent, then the variance among contrasts of the log-variances for one character should equal that for the other (i.e. H_o: $\sigma^2_{logVar(CV)} = \sigma^2_{logVar(DR)}$). One tests this hypothesis by correlating the sum of the log-variances for the two characters and their difference, across contrasts; a significant correlation indicates a significant difference in the two variances (Snedecor & Cochran 1980:190-1). Applying this test to the salamander data reveals no significant difference between the variance among contrasts for C-value and differentiation rate (Fig. 6b), and so we cannot reject the hypothesis that these two characters have the same exponent. Two points to note: like the previous test, this one is only approximate as log-variances are unlikely to be normally distributed (though the situation may be improved slightly as they will often be based on more than two values), and significant differences in variances may be due not only to differences in exponent (i.e. the variance due to time in figure 6a), but also, perhaps, to differences between characters in the heterogeneity of rates of evolution (i.e. the residual variation).

Phylogenetic inertia. Returning to the uses of data from molecular comparisons, or other information on times of divergence, one can ask whether the significant negative association between C-value and differentiation rate changes as a function of the age of the contrast. Such a change might be predicted by an hypothesis of phylogenetic inertia. This process occurs when changes in X result in changes in selection pressure on Y, but there has not been enough time for Y to respond completely, such that the population mean is lagging behind the optimum; the faster X changes, the more Y should be lagging. Thus an hypothesis of phylogenetic inertia would predict that the slope of the relation within contrasts should become shallower as one considers more recent contrasts. Of course, it is possible that differentiation rate is not responding directly to changes in C-value, but that they are each responding - in opposite directions - to changes in some other unknown variable Z; if so, then an association between the slope of differentiation rate on C-value within contrasts and the age of the contrast would suggest that they differ in their relative rates of response to Z - one is lagging more than another. As can be seen from figure 7, there is in fact no significant association. This result should not come as a great surprise: first, the mechanism relating C-value to differentiation rate is not thought to be evolutionary - increases in C-value do not select for decreases in differentiation rate - but rather biochemical, and thus immediate. Second, even for two variables related through natural selection, the selection coefficients or heritabilities would have to be quite low to show lags in contrasts over 4 million years old.

An analogous test can be devised in the absence of molecular data, though it will probably be weaker. Using a phylogeny one can often tell which of two contrasts is more recent, and then compare the slopes of those contrasts. There are 5 such pairs of contrasts to be made in the salamander phylogeny and 4 of them go against the hypothesis (Table 3).

These tests for phylogenetic inertia are very similar to tests for consistent changes in slope with taxonomic level (i.e. a consistent increase or decrease in slope as one calculates slopes for species within genera, genera within families, families within orders, etc. - e.g. Harvey & Mace 1982).

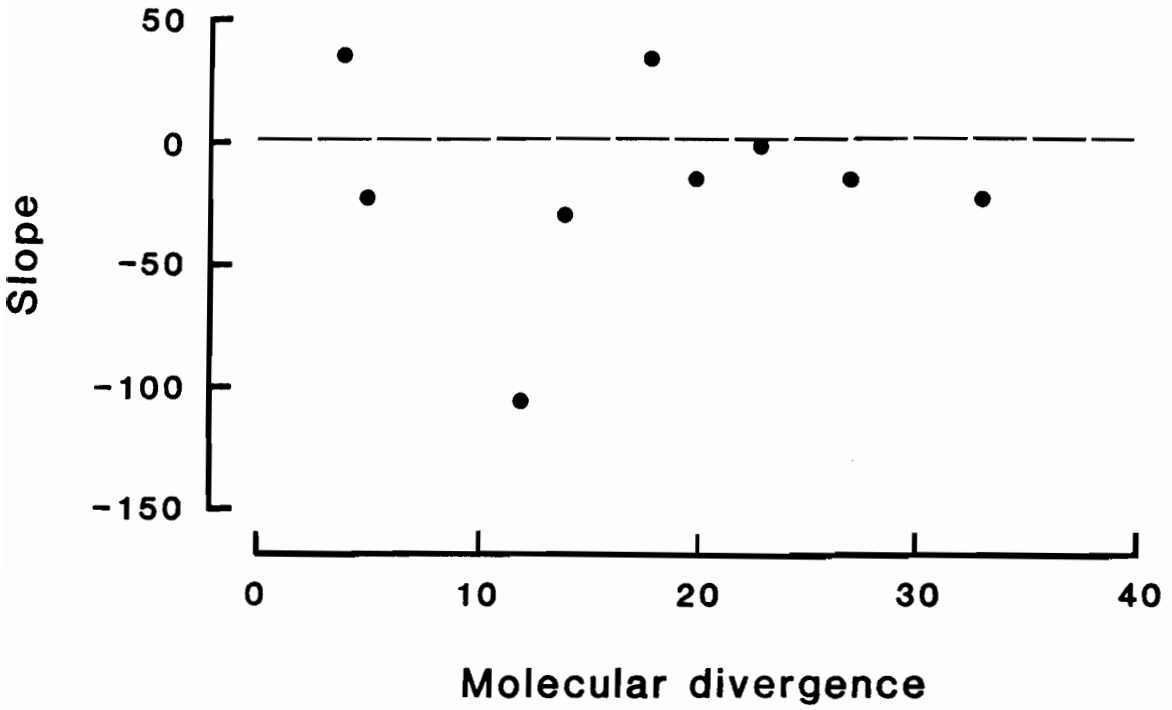

Figure 7. Test for phylogenetic inertia. As there is a negative correlation between differentiation rate and C-value, an hypothesis of phylogenetic inertia predicts a negative correlation between the slope of the relation within contrasts and the age of the contrast (i.e. older contrasts should have more negative slopes). Here age is estimated by molecular divergence and there is no indication of a trend: the slope of the association does not depend on the age of the contrast. Data from Tables 1 and 2.

Table 3. Sign test for phylogenetic inertia when data on times of divergence are not available. The null hypothesis is that the relative branching order of the contrasts is independent of the relative value of the slopes within contrasts; the hypothesis of phylogenetic inertia predicts that older contrasts will have more negative slopes. Thus, for example, we know from the phylogeny that contrast C is older than B, and so we predict that it should have a more negative slope, as indeed it does (-44.8 vs -15.3). However, contrast D is older than E, yet has a higher slope (-13.0 vs -24.5).

Predicted	Observed?
C < B	Yes
D < E	No
G < F	No
H < I	No
K < L	No

However, they are not to be confused with various tests of taxonomic similarity proposed by several other authors. Cheverud et al. (1985) estimate "phylogenetic inertia" with an autocorrelation coefficient and Derrickson & Ricklefs (1988) talk about phylogenetic constraints in terms of the slope and shape of a relationship differing in different taxa. However, these are measures of taxonomic similarity, akin to a nested ANOVA; as such they may (or may not) be useful (see also Bell 1989). The test described here is specifically for a trend in the slope of the relation with time. While phylogenetic inertia is one possible cause of taxonomic similarity, it is by no means the only, and the two concepts should not be synonymized.

OTHER USES FOR INDEPENDENT CONTRASTS

The method of independent contrasts is useful in comparative biology to deal with a specific problem: one wants to test a causal hypothesis relating X and Y, but there is a plausible process with no causal link between X and Y which nevertheless will routinely generate significant correlations between them. In interspecific comparisons this process is the sporadic branching of the evolutionary tree and the production of descendant phenotypes by modification of ancestral phenotypes. Similar statistical problems may be widespread. For example, correlations among individuals are very common in biology, yet close relatives are likely to be more similar than distant relatives. Those who study lakes regularly regress limnological variables on each other using lakes as data points, yet closely neighbouring lakes are presumably more similar than those far apart. In both cases, one approach to dealing with this underlying structure in the data is to use independent contrasts. Does the relationship hold within families? within regions?

SUMMARY

Interspecific correlation is a popular means of testing evolutionary and ecological theory. However, computer simulations by Raup & Gould (1974) demonstrate that statistically significant correlations can easily arise between two variables undergoing independent random evolutionary walks.

Indeed, significant associations are likely whenever closely related species are more similar than distantly related species, for then species with similar X-values are likely to be closely related, and thus have similar Y-values, independent of any causal mechanism relating X and Y. Here, I discuss an alternative test of association which avoids this problem and thus can provide a more powerful test of specific causal hypotheses than simple interspecific correlations.

The method involves two steps: first, dividing the data set into a series of independent replicate contrasts, and second, testing for a consistent association between changes in X and changes in Y. Several previous discussions of this method have defined the independent contrasts in terms of pairs of closest relatives or taxa at one level of the Linnean hierarchy (e.g. genera); here I suggest a novel method of recognizing independent contrasts in a known phylogeny. If contrasts are represented as a path joining the member species through the phylogenetic tree, then contrasts are independent if their paths do not meet at any point. For n species there are a maximum of n/2 phylogenetically independent contrasts, given a dichotomously branching phylogeny; multiple-branch points can be easily accommodated, though they reduce the total number of contrasts. Complications arise if contrasts must be extracted from a Linnean classification: if the taxa as represented in the data set are thought to be monophyletic then exactly analogous methods apply; however, if paraphyletic then the conservative solution is to use contrasts from only one level of the Linnean hierarchy. Each phylogenetically independent contrast contributes one degree of freedom to the subsequent test of association; any association which is significant by this test cannot be due to phylogenetic similarity. A sign test on the covariance of characters within contrasts provides a conservative nonparametric test. The method can be easily extended to test for an association between two qualitative variables, though in general one will need a larger data set in order to get the same number of contrasts.

While this test of association is purely ahistorical in nature and does not require data on times of divergence, such information, if available, can be used together with the phylogenetically independent contrasts in explicitly historical analyses. The relationship between phenotypic and molecular divergence can be used to look for evidence of 'punctuations'

associated with speciation and to test null models of evolutionary change (e.g. random walks). Further, if two phenotypic characters are found to be correlated, the the relationship between the slope of the association within contrasts and the age of the contrast can be used to test hypotheses of phylogenetic inertia. Similar analyses are also presented for the much more common instance of there not being any data on times of divergence other than the phylogeny, though the tests are correspondingly weaker.

All analyses are illustrated using Sessions & Larson's (1987) data on C-value and differentiation rate in plethodontid salamanders. The main conclusions are:

(1) Increases in C-value tend to be associated with decreases in differentiation rate, and vice versa, across 12 phylogenetically independent contrasts.

(2) There is no indication of punctuations in either C-value or differentiation rate at time of speciation.

(3) The evolution of C-value and differentiation rate is compatible with a null model of random walk; however, the tests have rather low power and more data will be necessary to provide a more precise test.

(4) There is no evidence of phylogenetic inertia in the relationship between C-value and differentiation rate.

Throughout the paper these methods are compared to various proposed alternatives. I conclude with several brief comments on the application of the method of independent contrasts beyond the problem of species comparisons.

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CHAPTER SIX

Losses to pathogens, pests, and competitors in agro-ecosystems

ABSTRACT

Antagonistic evolution by the biotic environment has been implicated in numerous biological phenomena, but there is as yet little quantitative information on species differences in susceptibility to various enemies and the correlates of these differences. We analyse estimates of crop losses due to viruses, bacteria and fungi, weeds, nematodes, and insects, relating them to taxonomic and ecological attributes of the crop. Crop losses due to different types of enemy are mostly independent of one another. There are no simple associations between level of damage and the taxonomic affiliation of the crop. Damage suffered by a crop is for the most part independent of area of cultivation, mode of propagation and breeding system; exceptions are positive correlations between area of cultivation and weed damage and between level of outcrossing and insect damage. Growth habit is a much better predictor: annuals suffer less damage due to viruses, weeds, insects and in total than perennial herbs and shrubs. This association between susceptibility and longevity does not apply to orchard trees, which tend to suffer less damage than annuals.

INTRODUCTION

The enemies of a population of organisms -- its predators, browsers, competitors, prey, and parasites -- form a distinctive component of the natural environment because they too are subject to evolution by natural selection. Such environmental evolution will often tend to be harmful to the population in question, and will elicit an evolutionary response, potentially leading to a continuous arms race (e.g. Dawkins & Krebs 1979; Vermeij 1987). This supposed contrariness of biotic environments has been implicated in the maintenance of genetic diversity within populations (Clarke 1979) and species diversity within communities (Janzen 1970), the evolution of sex (Jaenike 1978), and patterns in the fossil record of age-independent rates of extinction (Van Valen 1973) and increasing elaboration of weapons and armour through time (Vermeij 1987).

Despite this interest in the biotic component of environments, there seems to be little quantitative information on species differences in losses to various antagonists and even less on the correlates of these differences. In

this paper we analyse estimates of losses due to various parasites, pests, and competitors for crops grown in the United States. Both the agronomic and ecological literatures contain suggestions concerning the determinants of a species' susceptibility to losses; we wish to see whether these ideas are actually useful in constructing robust statistical generalizations. In particular, we focus on four potential correlates: area of cultivation, growth habit, mode of propagation, and breeding system.

We wish to stress from the outset that these data are derived from a very peculiar sort of ecosystem and it is not clear whether similar results will be found in more natural communities. Nevertheless, the American agro-ecosystem is to our knowledge the only public source of extensive information on losses to biotic agents. Such data should be interesting to academic population biologists both in its own right and as a basis of comparison for future studies.

Area of cultivation. Most previous attempts to describe the incidence of pathogens and pests and to relate observed differences to characteristics of the host species have focussed on the number of parasite species reported to attack the host species (e.g. Lawton & Schroder 1977; Strong & Levin 1979). This value is usually found to be correlated with the geographical range of the host (review in Strong et al. 1984). Yarwood (1962) reports a similar finding specifically for crops, with the number of recorded diseases correlating positively with the total value of the crop. Such findings suggest a positive correlation between percent loss of a crop and area of cultivation. On the other hand, it is possible that crops which suffer less damage will be more popular among farmers, and so one might predict a negative correlation between area of cultivation and average loss. Indeed, the cultivation of some crops is not possible in some areas of the United States due to excessive losses to parasites (U.S.D.A. 1965). Here we test these ideas by looking for an association between percent damage to a crop and area cultivated.

Growth habit. Parasites are often observed to do better on the host individual or population from which they were collected than on others of the same species (e.g. Caten 1974; Edmunds & Alstad 1978; Wainhouse & Howell 1983; Parker 1985; Karban 1989; but see Unruh & Luck 1987). Such results indicate that parasite populations on a host may evolve -- by natural selection or directed movement-- so as to circumvent the host's

defenses, and suggest that longer-lived hosts may suffer more damage. To test this idea we compare losses of annual and short-lived perennial crops. For completeness we also compare these to losses in orchard trees, though the very different horticultural practises make interpretation difficult.

Mode of propagation and breeding system. Many infectious diseases can be transmitted by vegetative propagation but not through seeds (see Parker 1987 for an example from a natural ecosystem; also Crocker 1939). Thus one might expect seed-propagated crops to suffer less damage due to infectious diseases than vegetatively-propagated crops. In addition, the conventional wisdom suggests that epidemics in agro-ecosystems are due in large part to the genetic homogeneity of modern crops (e.g. Adams et al. 1971; Anonymous 1972; Barrett 1981). Experiments comparing infection rates and insect abundances on mixtures and monocultures of susceptible and resistant plants tend to show levels on mixtures which are significantly lower than the mean of the monocultures (Risch et al. 1983; Burdon 1987:47). For insect pests, this effect of mixtures seems to be greater for annual crops than for perennial crops (Risch et al. 1983). Thus decreased genetic variability within a crop should be associated with increased levels of damage. Unfortunately, direct measures of genetic variance in farmers' fields are rare, and we have had to use the crop's breeding system as a substitute. The correspondence between genetic variance and breeding system is not exact -- surprisingly high levels of isozyme diversity have been found within varieties of barley, an inbreeder, and modern hybrid breeding techniques can eliminate much of the field variance of corn, an outcrosser (Simmonds 1962; Day 1973). Nevertheless, the assumption of a general trend between genetic variance and breeding system is probably valid, particularly in the 1950's when most of the loss data was recorded. Certain precedents also lead one to be optimistic: Stevens (1939) reports that more was written on the diseases of six inbred grain crops per unit value than on diseases of three outbred grains, and concludes they probably suffer more damage. Using a complicated measure of disease importance, he also indicated that vegetatively propagated crops were more likely to suffer from an "outstanding disease" than outcrossed seed crops, with inbred seed crops having an intermediate value (Stevens 1948). Here we compare losses in vegetative and seed propagated crops, and then compare inbred, mixed, and outbred seed crops.

DATA AND ANALYSIS

U.S.D.A. (1965) reports average losses in the continental United States for 1951-1960 due to insects, weeds, and infectious diseases. The latter were divided into viruses on the one hand, and bacteria and fungi on the other, mainly using U.S.D.A. (1960). Losses due to nematodes for 1970 were taken from Society of Nematologists (1971). Both compilations present annual losses in yield as a percent of the total crop. The estimates are of varying quality, some based on detailed surveys and records, others on expert judgements and informed opinions; all are based on actual losses in farmers' fields, after the application of pesticides and other conventional control measures. Note that losses in yield will tend to underestimate mean losses per individual plant if compensation occurs between neighbours. Also, annual losses will tend to underestimate lifetime losses in perennial crops.

After excluding greenhouse, ornamental, and hay crops, information was available for 99 crops distributed among 28 families and 9 classes (Appendix). The best represented families are the legumes (n=17) and the grasses (n=16). There are many missing values in the data set, and we could calculate total damage as the sum of all five components for only 28 crops.

Mean areas of cultivation in the United States for 1951-1960 were taken mostly from U.S.D.A. (1963). Areas of cultivation for 1962-1968 as reported by Society of Nematologists (1971) correlate very strongly with these values (r=0.92, n=41). Growth habits, modes of propagation, and breeding systems were taken mainly from Allard (1960), Simmonds (1976) and Hartmann & Kester (1983) (see Table 1).

Analyses of variance and correlations are calculated using each crop as an independent datum. In most cases each crop is a different species, though a few crops belong to the same species (e.g. Brassica oleracea: broccoli, cabbage, cauliflower, kale and Brussels sprouts; Lycopersicon esculentum: tomatoes, fresh market and tomatoes, processing). There is a growing appreciation that such analyses do not always provide the most rigorous tests possible: statistically significant associations may arise

Table 1. Classification of crops by growth habit, mode of propagation, and breeding system.

Propagation: Vegetative		Seed		
Breeding system:		Inbred	Mixed	Outcrossed
Growth habit				
Annual herb	3: Potato, Sweet potato, Garlic	21: Okra, Flax, Soybean, Dry, Lima and Snap beans, Cowpeas, Peanut, Dry and Green peas, Green pepper, Tomatoes (2), Tobacco, Eggplant, Endive, Lettuce, Oats, Barley, Rice, Wheat	11: Cotton, Cantaloup, Melon, Cucumbers (2), Watermelon, Squash, Sesame, Safflower, Sorghum (2)	16: Table and Sugar Beets, Spinach, Broccoli, Cabbage, Cauliflower, Kale, Brussels sprouts, Celery, Carrots, Crimson and Red clover, Rye, Corn, Sweet corn, Onions, Shallots
Perennial herb & shrub	11: Blue-, Cran-, Straw-, Black-, and Raspberries, Hops, Grapes, Mint, Artichokes, Sugar cane, Pineapple	0	0	9: Birdsfoot trefoil, Alfalfa, Alsike, Red and White clover, Smooth brome, Orchard-grass, Tall fescue, Timothy
Tree	17: Figs, Walnuts Dates, Filberts, Pecans, Apples, Peaches, Nectarines, Almonds, Apricots, Cherries, Plums/ Prunes, Pears, Oranges, Lemons, Grapefruit Tangerines	0	0	0

Note: 11 crops cannot be unambiguously classified.

between independently evolving characters if some pairs of species are more closely related than others (Raup & Gould 1974; Burt 1989). One may circumvent this problem by asking instead whether changes in one character tend to be associated with changes in the other (Felsenstein 1985; Burt 1989); unfortunately, our knowledge of crop phylogeny is too fragmentary and our measurements of potential correlates too crude to allow us to test such hypotheses. To reduce the probability that associations observed in the simple ANOVAs and correlations are due to phylogenetic similarity, we have repeated all statistical tests using generic means (Harvey & Mace 1982). As will be seen below, differences among families and higher taxonomic units are generally much smaller than those between species and genera within families.

The arcsin transform is used for estimates of damage, the logarithmic transform for areas of cultivation (Sokal & Rohlf 1981).

RESULTS

The mean total crop loss to biotic antagonists is 46% (Table 2). This value is quite evenly divided among four types: bacteria and fungi, weeds, nematodes, and insects. Though the mean reported damage due to viruses is one-fifth of these other types, it can be quite high (e.g. 25% on lupines) and may be grossly underestimated as our knowledge of viruses was relatively rudimentary 25 years ago.

There are no strong correlations between estimates of damage for the five different antagonists (Table 3). Weak but statistically significant correlations are observed between viruses and bacteria and fungi (positive) and weeds and nematodes (negative). There are no significant correlations among generic means (for all, $|r| \leq 0.27$ and $p > 0.1$).

Using the Linnean classification of crops and nested analysis of variance, one can partition the variance in losses among levels of the taxonomy (Table 4). In general, most of the variance is at the species and genus levels, though there is slightly more variance at higher levels for weeds and nematodes than for viruses, bacteria and fungi, and insects.

Table 2. Univariate statistics for crop losses expressed as percentages.

	n	Mean	Mean-s.d.	Mean+s.d.	Min.	Max.
Viruses	77	1.9	0.0	6.7	0.0	25.0
Bacteria & Fungi	77	10.5	5.3	17.3	1.4	28.0
Weeds	63	9.0	5.4	13.5	3.0	25.0
Nematodes	63	7.9	3.1	14.7	0.1	20.0
Insects	57	10.8	4.8	18.7	2.0	38.0
Total	28	45.9	33.2	58.9	25.5	86.0

Note: Statistics calculated on arcsin square root transformed data and then back-transformed.

Table 3. Correlation matrix of damage attributable to five antagonists.

	Bacteria & Fungi	Weeds	Nematodes	Insects
Virus	.277*(77)	.113 (50)	.088 (51)	.000 (56)
Bacteria & Fungi		.250 (50)	.094 (51)	.062 (56)
Weeds			-.369*(37)	.161 (37)
Nematodes				-.231 (45)

Note: Numbers in parentheses are sample sizes. * - $0.05 > p > 0.01$.

Table 4. Taxonomic distribution of variance in crop losses.

	Viruses (n=77)	Bacteria & Fungi (77)	Weeds (63)	Nematodes (63)	Insects (57)	Total (28)
Class	5	0	0	16	3	13
Subclass	0	0	0	0	8	0
Family	4	2	44	20	0	0
Genus	38	30	36	0	77	66
Crop (incl. error)	52	69	20	64	12	22

Note: Values indicate the percent of total variance accounted for at each taxonomic level as estimated by nested analysis of variance.

There is no significant correlation between area cultivated and total damage (Fig. 1). If we consider the five types of antagonist simultaneously, the type x area interaction approaches statistical significance ($F_{4,294}$=2.3, p=0.06). Apparently this is due to a weak but highly significant positive correlation between weed damage and area of cultivation (Fig. 1). No other component of damage correlates with area of cultivation. Using generic means, again weed damage is the only type to correlate with area of cultivation (r=0.366, n=40, p=0.02).

Perennial herbs and shrubs suffer significantly more total damage than annuals (69 vs 44%, $F_{1,23}$=15.4, $p < 0.001$). Considering the five types of loss simultaneously, there is a very highly significant type x habit interaction ($F_{4,261}$=6.3, $p < 0.001$). Annuals suffer significantly less damage from viruses, weeds, and insects; for bacterial and fungal damage the difference is in the same direction but is not statistically significant (Fig. 2). Analyses based on generic means give similar results. Analysis at the level of crops suggests annuals suffer more damage from nematodes than do perennial herbs and shrubs (Fig. 2), but this difference is not statistically significant in the analysis of generic means ($F_{1,34}$=2.7, p = 0.11). The overall association of increased damage with longevity does not apply to orchard trees, which tend to suffer even less damage than annuals (Fig. 2).

There is no significant difference between vegetative and seed-propagated crops in total damage (53% vs 44%, $F_{1,26}$=2.3, p=0.14). Considering the five types of antagonist simultaneously, there is no significant type x mode interaction ($F_{4,312}$=0.6, p=0.7). None of the five types individually show a significant difference between vegetative and seed propagated crops (Fig. 3). Using generic means, virus and total damage merely approach formal significance a little more closely ($F_{1,48}$=2.7, p=0.11 and $F_{1,22}$=3.2, p=0.09 respectively).

Finally, to test for an association between outcrossing and losses, values of 1, 2, and 3 were assigned to inbred, mixed and outbred crops respectively and Spearman rank correlations were calculated. There is no correlation between level of outcrossing and either total damage (43 vs 48 vs 43%, r_s=0.04, p=0.8) or any of its components except insect damage (Fig. 3). Outcrossed seed crops tend to have more damage due to insects than do inbred crops. The same results follow from analysis of generic means.

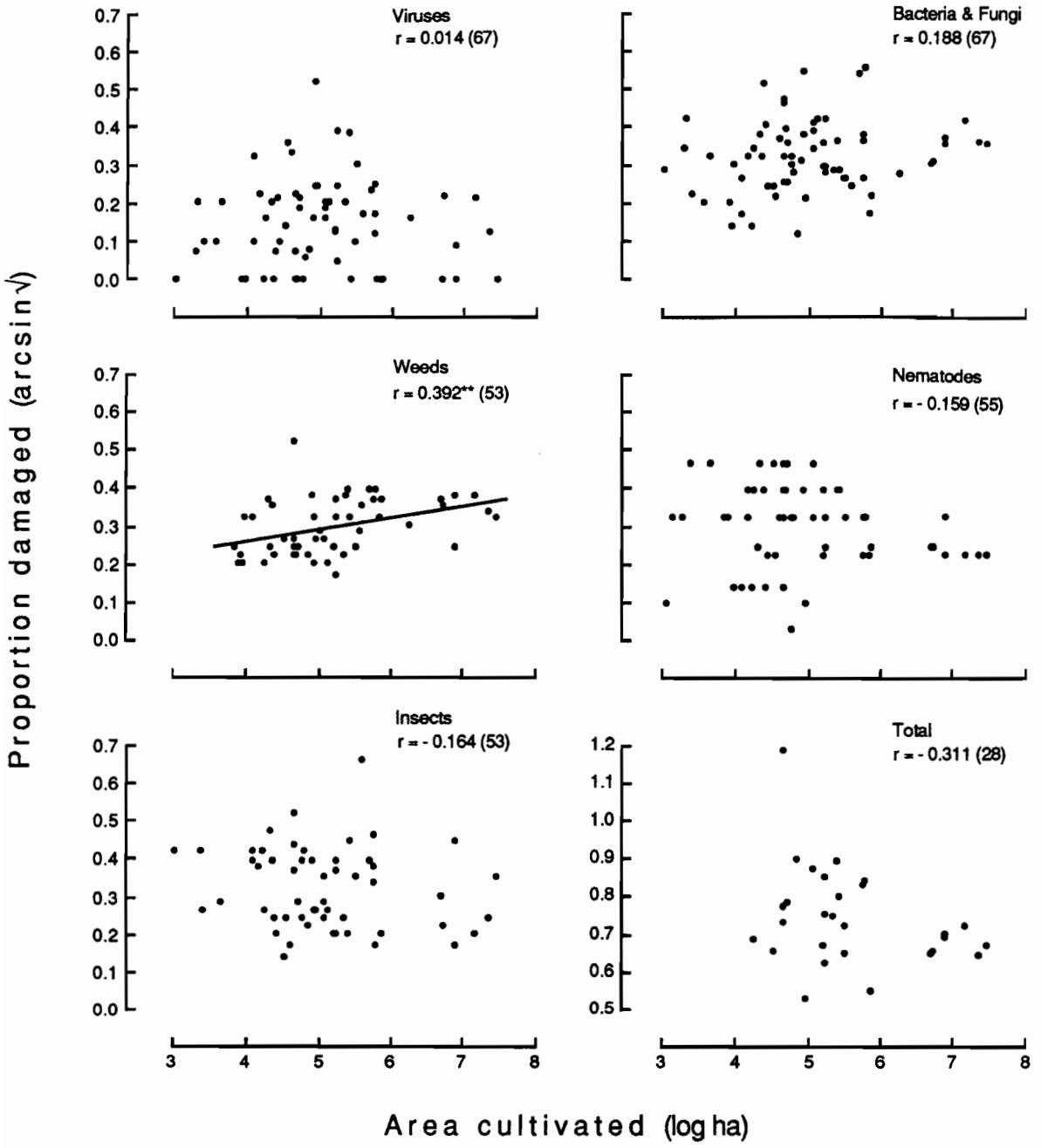

Figure 1. Crop losses as a function of area of cultivation. Proportions have been arcsin transformed and areas have been log transformed. ** - $0.01 > p > 0.001$.

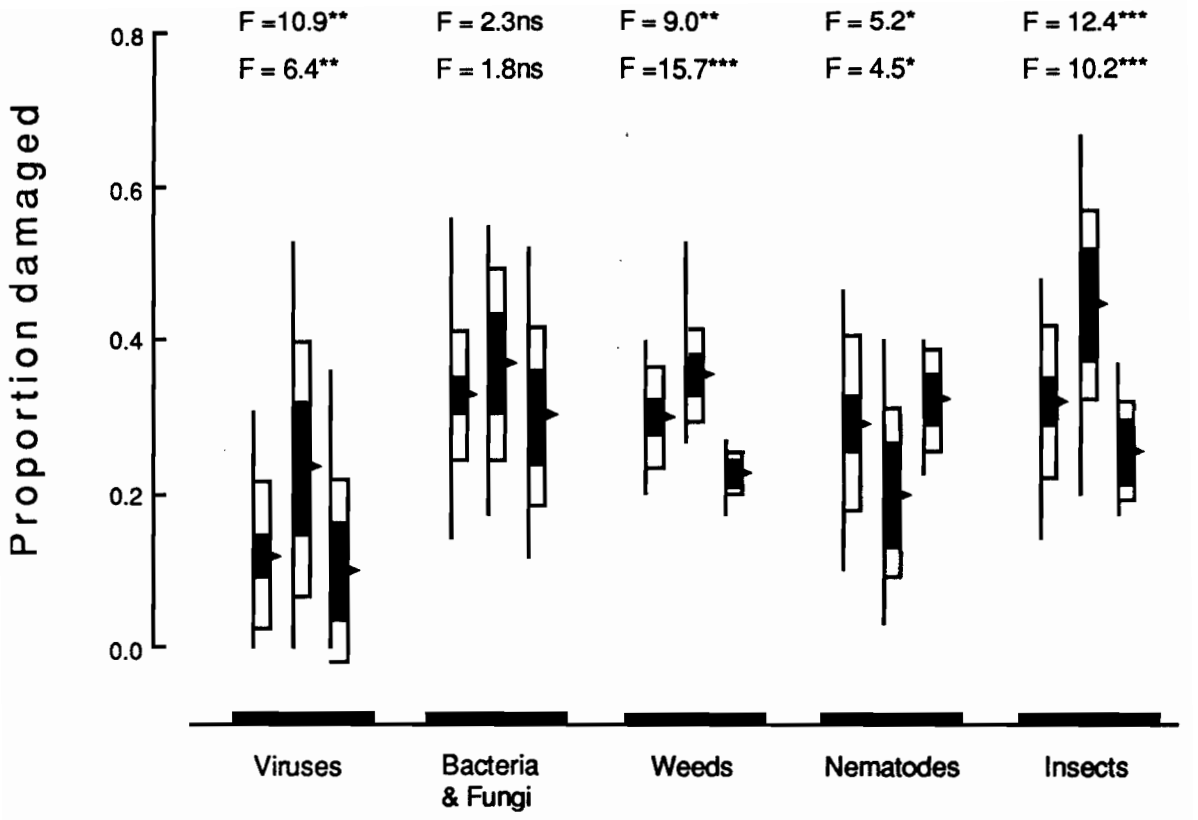

Figure 2. Crop losses as a function of growth habit. The losses suffered by annuals, perennial herbs and shrubs, and trees are shown for each of five types of damage. Dicegrams show mean, 2 SE, SD, and range. For each type of damage the first F-ratio tests for a difference between annuals and perennial herbs and shrubs, the second for differences among all three growth habits. * - $0.05 > p > 0.01$; ** - $0.01 > p > 0.001$; *** - $p < 0.001$.

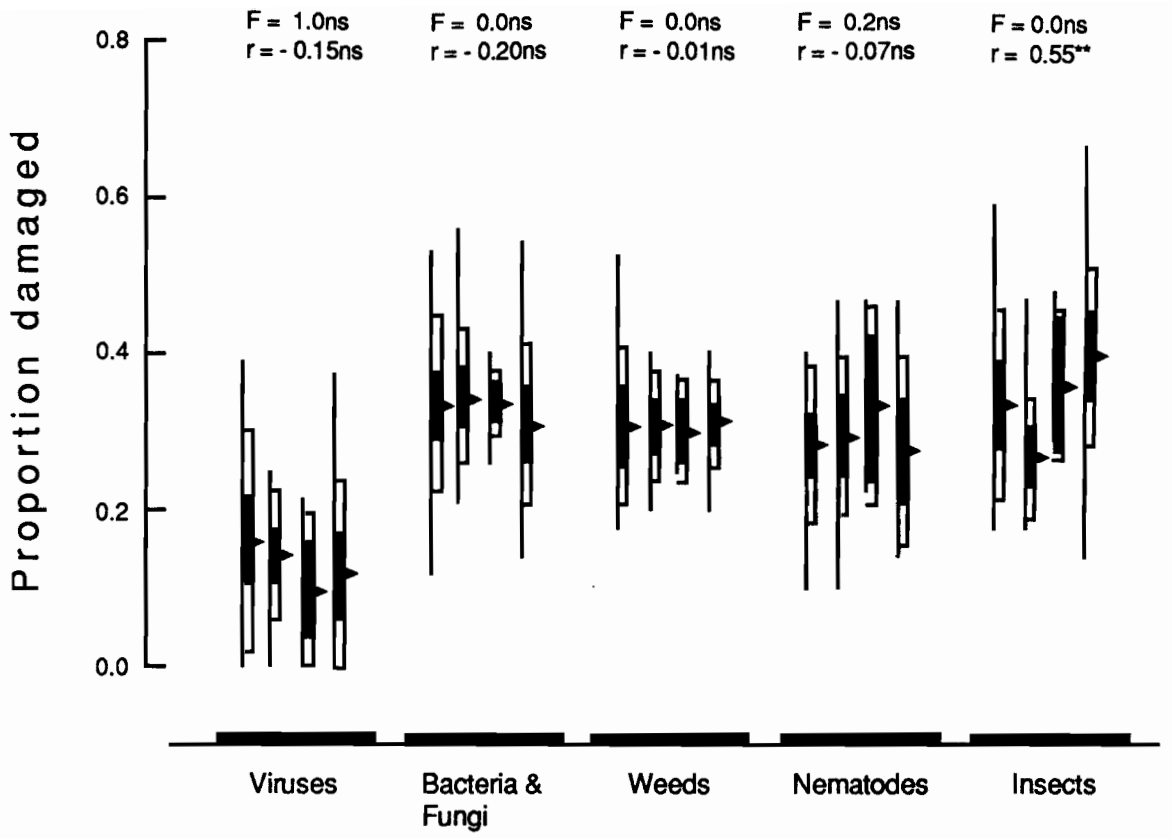

Figure 3. Crop losses as a function of mode of propagation and breeding system. The losses suffered by vegetatively propagated crops and inbred, mixed and outcrossed seed crops are shown for each of five types of damage. Dice-grams show mean, 2 SE, SD and range. For each type of damage the F-ratio tests for a difference between vegetative and seed propagated crops and the Spearman rank correlation coefficient describes the correlation between damage and outcrossing within the seed crops. *** - $p < 0.001$.

DISCUSSION

Bacteria and fungi, weeds, nematodes, and insects are each responsible for average crop losses of roughly 10%, even after all the standard control measures have been applied. Viral damage is reported to be much lower, though this may have been an underestimate. The five components of damage are for the most part independent of one another, indicating that susceptibility to different enemies is likely to be determined by different characteristics of the crop. For each of these components of loss, most of the variance among crops is at a low taxonomic level, with little indication of consistent differences even among Linnean families. Three of the four ecological variables were also of little use in deriving robust statistical generalizations, as we now discuss.

Area of cultivation. Damage due to most types of antagonist and total damage were independent of the area of cultivation of the crop. These results indicate that while widespread crops may have more species of antagonists over their entire range (Yarwood 1962), the losses suffered in any one area do not depend on whether the crop is grown in another. Only weed damage was found to be correlated with area of cultivation; it is not clear to us what distinguishes weeds from the other antagonists to give this result. We also note that a crop's susceptibility to damage does not seem to determine the area over which it is grown, at least in any simple manner. It is conceivable that farming practise tends to extend the area of cultivation of a crop until, with rising disease levels, its profitability falls to the average of all crops. This would tend to weaken any correlation between disease level and area of cultivation that would appear in a randomized experiment. More complicated agronomic models are beyond the scope of this paper.

Growth habit. Perennial herbs and shrubs suffer more damage from viruses, weeds, and insects, than do annuals, and suffer more total damage. This is the pattern predicted by theory. However, there was no significant difference for bacterial and fungal damage and the trend may be reversed for nematodes (though there was no significant difference using generic means). Note that these data are for annual losses; differences in lifetime losses will tend to be even greater due to the compounding of growth and mortality losses in perennials. These results support the idea that longer-

lived organisms are likely to have more problems dealing with their biotic environment (e.g. Hamilton 1982). However, this trend does not extrapolate to orchard trees, which tend to suffer even less damage than annuals. We suggest this is because they start life in the field as a branch grafted onto a more-or-less established rootstock and can receive more individual horticultural care. Diseased rootstocks and trees are likely to be removed, maintaining low levels of damage in the crop.

Mode of propagation. Vegetatively propagated crops have higher mean levels of virus and total damage than seed propagated crops, but this difference is not statistically significant. One interpretation is that the attempts by breeders to establish disease-free lines for cuttings have been successful to the point that they have reduced field losses to the level of seed propagated crops.

Breeding system. Using various crude measures of susceptibility, Stevens (1939, 1948) finds that vegetatively propagated crops and inbred seed crops suffer more damage from disease than do outbred seed crops and concludes that genetic homogeneity leads to increased potential for losses. Since then there has been widespread agreement in the agronomic literature on this point (e.g. Adams et al. 1971; Anonymous 1972; Barrett 1981). However, we found no evidence of such an effect. As already noted, there were no significant differences between vegetative and seed-propagated crops. In addition, there were no consistent differences between inbred and outbred crops, the only significant association being a positive correlation between insect damage and outcrossing.

How might one account for this apparent discrepancy? One possibility is that genetic homogeneity has little effect on the mean damage, but rather increases the year-to-year variance in damage, and thus the probability of there being an epidemic. Experimental work has shown that crop mixtures often result in more stable yields than the component monocultures, and this effect on the variance is generally more striking than any effect on mean yield (Marshall & Brown 1973; G. Bell, unpublished MS). To test specifically for the effect of mixtures on losses to parasites, it is necessary to compare mixtures and component monocultures both in the presence and the absence of the parasite. Unfortunately, few such experiments have been performed (Dempster & Coaker 1974; Wolfe & Barrett 1980; Jeger et al. 1981) and no clear conclusions have emerged.

CONCLUSIONS

Crop losses due to different types of enemy are mostly independent of one another. There are no simple and robust associations between damage and the taxonomic affiliation of the crop, its area of cultivation, its mode of propagation or its breeding system. Perennial herbs and shrubs suffer more from most types of damage than annuals, but this trend does not extrapolate to orchard trees. It remains an open question whether these results will be found to apply to more natural ecosystems. Information on losses to insect herbivores is accumulating in experiments with controlled application of insecticides (references in Brown et al. 1987 and Crawley 1989), but there seems to be little comparable work as yet using fungicides or nematicides.

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APPENDIX

Raw data used in analysis of crop losses. Classification follows Mabberley (1987). Area of cultivation in hectares, log transformed. Habit: 1-annual, 2-perennial herb and shrub, 3-tree. Propagation: 1-vegetative, 2-selfed seed, 3-mixed seed, 4-outcrossed seed. Damage expressed as a percent of the total crop. Nematode and insect losses do not include indirect damage due to transmission of viruses. Note that the nematode estimates do not distinguish between the different types of cucumber, citrus, tomato and sorghum crops.

Class / Subclass / Family / Genus / Crop						Log Area (ha)	Habit	Prop	Percent Damage					
									Vir	B&F	Weed	Nem	Insect	Total
1	1	1	1	1	Avocados	3.838	3	.	.	.	6	10	.	.
1	2	2	2	2	Hops	4.093	2	1	10	3	10	.	15	.
1	2	3	3	3	Figs	4.163	3	1	.	.	.	15	.	.
1	2	4	4	4	Pecans	.	3	1	0	21	.	5	12	.
1	2	4	5	5	Walnuts	4.690	3	1	0	15	5	15	.	.
1	2	5	6	6	Filberts	3.927	3	1	0	4	.	.	.	.
1	3	6	7	7	Table beets	3.955	1	4	0	2	4	.	.	.
1	3	6	7	8	Sugar beets	5.519	1	4	9	7	6	10	12	44
1	3	6	8	9	Spinach	4.419	1	4	4.5	15.5	.	2	4	.
1	4	7	9	10	Okra	.	1	2	.	.	.	5	.	.
1	4	7	10	11	Cotton	6.874	1	3	0	12	6	5	19	42
1	4	8	11	12	Cantaloups	4.711	1	3	3.5	12.5	6	20	8	50
1	4	8	11	13	Melon	3.643	1	3	4	10	.	20	8	.
1	4	8	11	14	Cucumbers-fresh	4.316	1	3	4	14	6	>20<	21	>61.4
1	4	8	11	15	Cucumbers-pickling	4.709	1	3	4.5	6.5	6	.	.	
1	4	8	12	16	Watermelons	5.194	1	3	1.5	8.5	6	5	.	.
1	4	8	13	17	Squash	.	1	3	.	.	.	5	.	.
1	4	9	14	18	Broccoli	4.214	1	4	0	2	.	2	17	.

1	4	9	14	19	Cabbage	4.786	1	4	0.3	7.7	.	10	17	.
1	4	9	14	20	Cauliflower	4.083	1	4	1	7	.	2	17	.
1	4	9	14	21	Kale	3.012	1	4	0	8	.	.	17	.
1	4	9	14	22	Brussels sprouts	3.359	1	4	.	.	.	20	17	.
1	4	10	15	23	Blueberries	.	2	1	1	13	20	1	.	.
1	4	10	15	24	Cranberries	3.962	2	1	0	9	10	2	.	.
1	5	11	16	25	Strawberries	4.647	2	1	5	21	25	10	25	86
1	5	11	17	26	Apples	5.224	3	1	0.2	7.8	3	10	13	34
1	5	11	18	27	Peaches	5.194	3	1	1.7	12.3	6	15	4	39
1	5	11	18	28	Nectarines	3.112	3	1	.	.	.	10	.	.
1	5	11	18	29	Almonds	4.649	3	1	0.5	6.5	7	15	.	.
1	5	11	18	30	Apricots	4.440	3	1	1	6	.	5	.	.
1	5	11	18	31	Cherries	4.598	3	1	10.7	13.3	.	10	3	.
1	5	11	18	32	Plums/prunes	4.759	3	1	0	10	.	10	6	.
1	5	11	19	33	Pears	4.557	3	1	12.3	4.7	.	5	6	.
1	5	11	20	34	Blackberries	.	2	1	9.4	24.6	.	2	31	.
1	5	11	20	35	Raspberries	.	2	1	12.4	25.6	.	2	23	.
1	5	12	21	36	Lespedeza	5.381	2	.	.	.	14	.	.	.
1	5	12	22	37	Soybeans	6.887	1	2	0.8	13.2	14	10	3	41
1	5	12	23	38	Dry beans	5.765	1	2	3	14	13	5	20	55
1	5	12	23	39	Lima beans	4.662	1	2	0	10	6	20	13	49
1	5	12	23	40	Snap beans	5.067	1	2	4	16	7	20	12	59
1	5	12	24	41	Cowpeas	5.473	1	2	1	7	.	.	.	.
1	5	12	25	42	Peanuts	5.790	1	2	0	28	15	10	3	56
1	5	12	26	43	Birdsfoot trefoil	.	2	4	.	.	12	.	.	.
1	5	12	27	44	Dry peas	5.062	1	2	2.5	11.5	.	.	6	.
1	5	12	27	45	Green peas	5.239	1	2	6	17	10	10	4	47
1	5	12	28	46	Alfalfa	5.596	2	4	3	6	12	.	38	.
1	5	12	29	47	Sweetclover	4.938	2	.	.	.	10	.	.	.
1	5	12	30	48	Alsike clover	4.315	2	4	.	.	13	6	.	.
1	5	12	30	49	Crimson clover	4.917	1	4	2.5	9.5	14	.	15	.
1	5	12	30	50	Red clover	5.688	2	4	5.5	26.5	15	.	15	.
1	5	12	30	51	White clover	.	2	4	13	11	12	.	17	.
1	5	12	31	52	Lupines	4.933	2	.	25	27	10	.	.	.
1	5	13	32	53	Tung trees	.	3	.	0	2.1	.	.	.	.
1	5	13	33	54	Castorbeans	.	.	4	0	11	.	.	.	.

1	5	14	34	55	Grapes	5.399	2	1	14.1	12.9	15	15	4	61
1	5	15	35	56	Flax	6.236	1	2	2.5	7.5	9	.	.	.
1	5	16	36	57	Oranges	5.345	3	1	4	8	5		6	
1	5	16	36	58	Lemons	4.378	3	1	0.5	24.5	5		6	
												15		36.8
1	5	16	36	59	Grapefruit	4.843	3	1	0.6	1.4	5		5	
1	5	16	36	60	Tangerines	3.908	3	1	.	.	5		.	
1	5	17	37	61	Celery	4.161	1	4	5	10	.	10	14	.
1	5	17	38	62	Carrots	4.509	1	4	2	6	7	20	2	37
1	6	18	39	63	Green peppers	4.239	1	2	2.5	11.5	4	15	7	40
1	6	18	40	64	Tomatoes-fresh	4.941	1	2	6	14	4		7	
												>15<		>46.5
1	6	18	40	65	Tomatoes-processing	5.122	1	2	4	17	4		7	
1	6	18	41	66	Tobacco	5.759	1	2	1.4	6.9	.	5	11	.
1	6	18	42	67	Eggplant	3.276	1	2	0.5	11.5	.	.	.	.
1	6	18	42	68	Potato	5.753	1	1	6.2	12.8	.	10	14	.
1	6	19	43	69	Sweet potato	5.071	1	1	3.5	14.5	.	10	8	.
1	6	20	44	70	Mint	4.368	2	1	0	10	12	.	15	.
1	6	21	45	71	Olives	3.886	3	.	.	.	4	10	.	.
1	6	22	46	72	Sesame	.	1	3	0	11	12	.	.	.
1	6	23	47	73	Coffee	.	3	.	.	.	.	5	.	.
1	6	24	48	74	Safflower	.	1	3	0.2	11.8	13	.	.	.
1	6	24	49	75	Artichokes	3.554	2	1	1	4	.	.	.	.
1	6	24	50	76	Escarole/endive	3.388	1	2	1	5	.	.	7	.
1	6	24	51	77	Lettuce	4.946	1	2	6	4.5	7	1	7	25.5
2	7	25	52	78	Dates	3.260	3	1	.	.	.	10	.	.
2	8	26	53	79	Oats	7.142	1	2	4.6	16.3	14	5	4	43.9
2	8	26	54	80	Smooth brome	.	2	4	.	.	7	.	.	.
2	8	26	55	81	Orchardgrass	.	2	4	.	.	8	.	.	.
2	8	26	56	82	Tall fescue	.	2	4	.	.	9	.	.	.
2	8	26	57	83	Barley	6.706	1	2	4.8	9.2	12	6	5	37
2	8	26	58	84	Ryegrass	5.568	.	4	.	.	8	.	.	.
2	8	26	59	85	Rice	5.863	1	2	0	4.7	13	6	4	27.7
2	8	26	60	86	Timothy	5.027	2	4	.	.	8	.	.	.
2	8	26	61	87	Kentucky bluegrass	.	2	.	.	.	10	.	.	.
2	8	26	62	88	Sugarcane	5.223	2	1	14.5	8.5	13	6	15	57

2	8	26	63	89	Rye	5.829	1	4	0	3	10	5	.	.
2	8	26	64	90	Sorghum-grain	6.691	1	3	0	8.8	13	>6<	9	>36.9
2	8	26	64	91	Sorghum-sweet	.	1	3	0	15	13		.	
2	8	26	65	92	Wheat	7.350	1	2	1.6	12.4	11	5	6	36
2	8	26	66	93	Corn	7.443	1	4	0	12	10	5	12	39
2	8	26	66	94	Sweet corn	5.414	1	4	0	8	10	15	19	52
2	9	27	67	95	Pineapple	.	2	1	.	.	.	5	.	.
210	28	68	96		Onions	4.663	1	4	0	20	5	2	18	45
210	28	68	97		Shallots	3.312	1	4	4	17	.	.	.	.
210	28	68	98		Garlic	3.032	1	1	.	.	.	1	.	.
210	28	69	99		Asparagus	4.776	2	.	0	9	.	0.1	15	.

CHAPTER SEVEN

Tests of sib diversification theories of outcrossing in Impatiens capensis: Effects of inbreeding and neighbour relatedness

ABSTRACT

Several models of the evolution of genetic systems posit very strong frequency-dependent selection acting on small spatial scales; in such circumstances a genetically diverse sibship outperforms a genetically uniform sibship, and genes for mixis may spread in a population. The mechanisms most commonly invoked to generate this sort of selection are resource competition and parasite transmission. We describe a greenhouse experiment designed to test these ideas, using the annual herb Impatiens capensis. Plants were potted in pairs; the genetic variance within pots was manipulated by using progeny from either inbred or outcrossed parents and by using either full sibs or unrelated individuals. Treatment combinations designed to increase genetic diversity resulted in greater phenotypic variance in both morphology and production, though not in the density of spider mites or whiteflies. Despite evidence of resource limitation, there was no effect of genetic diversity on productivity, nor was there an effect on infestation. These results support neither the sib competition nor the sib contagion theories of outcrossing.

INTRODUCTION

One obvious consequence of sexual reproduction is the genetic diversification of the offspring from a single female, and several theorists have singled out this effect as a likely candidate for the primary function of sex. Proposed benefits of sib diversification include a partitioning of resources and a reduction in parasite transmission (Bell 1982, 1985; Price & Waser 1982; Tooby 1982). Both mechanisms have been thought to generate frequency-dependent selection on small spatial scales, which results in greater production in genetically diverse sibships than in genetically uniform sibships. These ideas have also been developed in population genetic models without family structure but which assume reproductive isolation between sexual and asexual populations (Bell 1982; Case & Taper 1986). They may also be applied to the evolution of outcrossing (Waller 1980, 1984; Schmitt & Ehrhardt 1987).

The main effect of extended inbreeding is to increase the total genetic variance in a population and to change the partitioning of that

variance such that more of it lies between families and less within families (Falconer 1981). To be precise, with simple additive gene action,

$$V(TOT) = (1 + F)\, V_G \text{ , and}$$

$$V(FS \in FAM)_t = (1 - F_{t-1})\, V_G / 2$$

where V(TOT) is the total genetic variance, V(FS $\in$ FAM) is the variance of full sibs within families, F is Wright's inbreeding coefficient, V_G is the genetic variance in a baseline random mating population, and t is the generation. Note in particular that the variance among full sibs within families depends on the inbreeding coefficient of the parents. Thus one cannot compare the productivity of inbred and outcrossed progeny from the same parent as a test of the sib diversification hypothesis if the outcrossed progeny might be full sibs. Indeed, if one allows for dominance in gene action, then inbred progeny may be even more variable than outcrossed progeny of the same parent, as emphasized by Mitchell-Olds & Waller (1985) and McCall et al. (1989). However, because genes for inbreeding will over generations come to predominate in inbred individuals, they will also become associated with small variances within families, even with dominance. It follows that the sib diversification theory of outcrossing is best tested in an experiment spanning at least three generations in which one compares the progeny of parents themselves derived from either inbred or outbred crosses. Here we describe such an experiment. The subjects were either from inbred or outcrossed parents and were planted either with full sibs or with unrelated individuals. The key response variables measured were total production and total infestation by two common greenhouse pests, spider mites and whiteflies.

NATURAL HISTORY

Impatiens capensis (Balsaminaceae), or jewelweed, is an erect annual forest herb. Each individual may produce two types of flowers: small cleistogamous (CL) flowers which never open and are therefore obligately self-fertilized, and showy chasmogamous (CH) flowers which open and can potentially outcross. CH flowers are pollinated by bees or hummingbirds.

Outcrossing is promoted by the fact that the stigma is only available for pollination after the androecium (pollen-producing organ) has fallen off; Waller and Knight (1989) report mean outcrossing rates of 0.3 to 0.7 for CH flowers. The proportion of seeds produced by CH flowers increases with total fecundity (Waller 1980). Seeds are dispersed from the parent generally no more than 2m via explosively dehiscent seed capsules. All viable seeds germinate the next spring -- there is no seed bank. Populations can suffer considerable damage from various insect herbivores, which tend to be host-specific (Schemske 1978).

The two-spotted spider mite (Tetranychus urticae, Tetranychidae, Acariformes) and the greenhouse whitefly (Trialeurodes vaporariorum, Aleyrodidae, Homoptera) are cosmopolitan greenhouse pests capable of considerable damage. For I. capensis specifically, Mitchell-Olds & Waller (1985) report that whiteflies were responsible for 23% mortality one year in the greenhouse, though mortality was negligible in other years. The spider mites are haplo-diploids, while both haplo-diploid and wholly parthenogenetic races of whiteflies are known (Helle & Sabelis 1985; Suomalainen et al. 1987). The generation time of spider mites can be as short as 10 days (Helle & Sabelis 1985), while that of whiteflies is about 30 days (Nelson 1978).

METHODS

The study extended over three years (Fig. 1). In the spring of 1987, seedlings were collected from a small natural population of I. capensis at Mont St. Hilaire, Quebec. This population is likely to be highly inbred both because it is isolated from other populations and because very few chasmogamous flowers are ever produced in it. The seedlings were transplanted into pots in a small experimental garden; on reaching maturity a sample of 16 individuals were moved to an insect exclosure. These were the grandparents of our experimental subjects, half of whom were descended from outcrossed CH seed produced by hand pollination and half from CL seeds. (A small number of experimental subjects [12] were also derived from CL seeds collected from 6 plants left in the garden.) These seeds were left to over-winter and grow the next year (1988) in the outdoor garden. Positions were not randomized and plants from CL seeds

were exposed to more sunlight and were generally smaller than those derived from the controlled matings. CL seeds from these plants were stored on damp filter paper at approximately 4°C and planted the next year (1989) in a climate-controlled greenhouse chamber in the McGill University Phytotron.

Upon germination (beginning day 0), seedlings were placed in 5 cm peat pellets. Established seedlings were then placed in 13 cm pots of "Promix", together with either a full sib or an unrelated individual from the same type of parent (outcrossed or inbred). Pots were arranged in blocks of four in the greenhouse, each block comprising a pair of sibs from inbred parents, a pair of sibs from outcrossed parents, a pair of unrelated individuals from inbred parents, and a pair of unrelated individuals from outcrossed parents. Because of a shortage of individuals from CL parents, 4 of the 22 blocks were not perfectly balanced. Plants were misted and fertilized as necessary with Hoaglands solution (version 2 -- Dunn & Arditti 1968).

Measurements. We measured plant height and diameter at the first node on days 39 and 75 and measured plant height again on day 116. Plants were harvested and processed by blocks when they began to senesce (days 153-169). To measure the incidence of adult whiteflies, pots were gently placed on an open garbage bag and the sides of the bag were quickly drawn up, around, and over the two plants. A ball of cotton soaked with ether was then placed in the plastic bag, which was left sealed for at least an hour. The plants were then removed and the inside of the bag examined for whiteflies. Some whiteflies escaped detection by this method; thus the reported values are likely to underestimate the true values. For each plant we then measured three consecutive internode lengths, starting at the bottom-most node; the total number of nodes; the minimum diameter at the first internode; and the maximum diameter at the second node. "Nodiness" is the difference between these two diameters. All leaves on every fourth branch were then removed and either examined immediately under a dissecting microscope or first pressed and examined later. We recorded the number of spider mites and immature whiteflies (including empty pupal cases) on the bottom of each leaf. The remaining above-ground portion of the plant was oven dried and weighed. Final height and dry weight are used

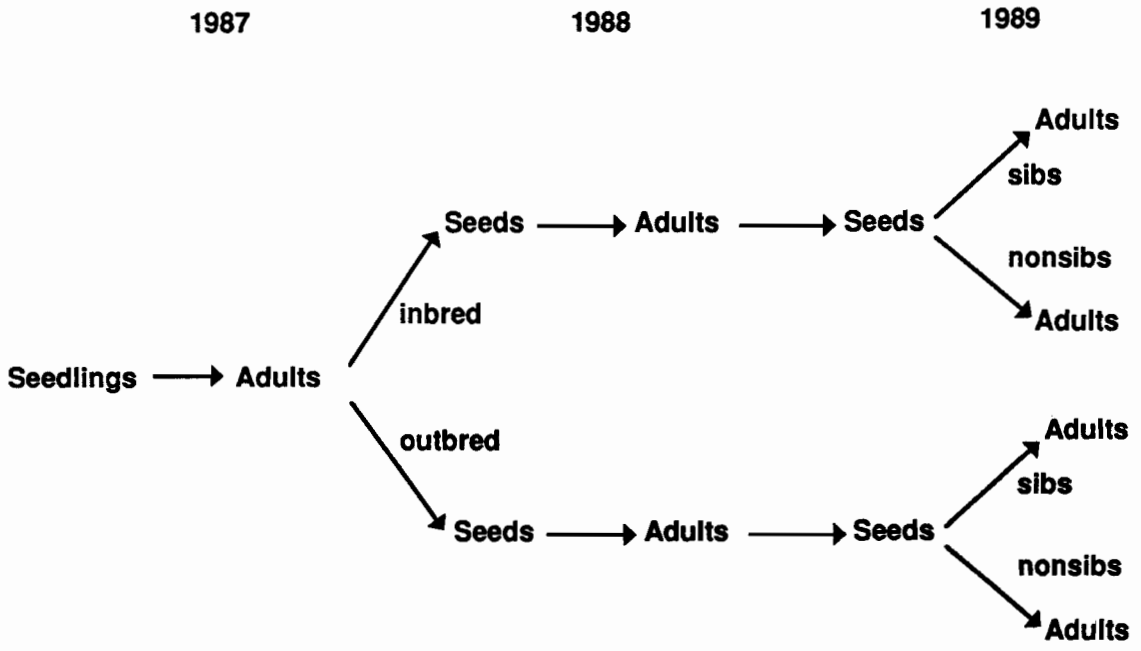

Figure 1. Schematic diagram showing the derivation of our subjects and experimental design. Individuals are from either inbred or outbred grandparental crosses and are grown with either a full sib or an unrelated individual from the same type of grandparental cross.

as measures of production; previous work in an experimental garden found that both were highly correlated with total lifetime fecundity (r=0.88 and 0.93 respectively, n=40; calculated from data in Smit 1986). We also attempted to eliminate maternal effects on final production by calculating residual height and dry weight, having removed the linear effect of height at day 39 (r^2=0.47 and 0.23 respectively, n=176).

Analysis. The rationale for the experimental treatments was to manipulate the genetic variance between the two plants sharing the same pot. The effect of putting full sibs together in a pot is to decrease the genetic variance. The effect of using progeny from outcrossed parents as opposed to inbred parents is to increase the variance between siblings but to decrease it between nonsibs (see equations above). Thus, the four treatment combinations, in order of increasing genetic variance, are (1) sibs of inbred parents; (2) sibs of outcrossed parents; (3) nonsibs of outcrossed parents; (4) nonsibs of inbred parents. The simplest analysis is the rank correlation between the response variable of interest and the treatment, with the four treatments being given values of 1 to 4, in order of increasing genetic variance. We also present the analyses of variance. Note that because the effect of outcrossing on genetic diversity in sibs is opposite to that in nonsibs, it is appropriately tested by the interaction of relatedness with degree of parental outcrossing (Rel*Out). Outcrossing also increases individual heterozygosity, which is tested by the main effect of outcrossing in the ANOVA. Although it would be possible to interpret this effect in terms of developmental homeostasis or heterosis, in our experiment the parental type is confounded with the parental environment, as inbred parents were in a more stressful environment (see above). To emphasize the fact that the main interest here is on the effect of outcrossing on genetic variance, we have chosen to present the interaction term before the outcrossing main effect in our tables of results; none of the mean squares are changed by this. Finally, note that the appropriate unit of analysis is the pot, except where indicated.

RESULTS

Phenotypic variances. To test whether the manipulations of genetic variance had an effect on phenotypic variance, we calculated the range within pots for each character. For all morphological and production characters there is a positive correlation between phenotypic and genotypic variance, which is statistically significant in many cases (Table 1). The analyses of variance tend to show the separate effects of relatedness and outcrossing in the predicted direction, sometimes significant. These results indicate the existence of genetic variance in the population for morphological and production characters. Note, however, that there is no genetic variance in either spider mite or whitefly infestation.

Resource limitation. To test whether resources in pots were limiting we compared the variance of plant size within and among pots by single classification ANOVA. At the beginning of the growing season there were highly significant differences in plant size among pots (Fig. 2). These differences declined with time, such that at time of harvest there was significantly less variance among pots than would be expected based on the variance within pots. These results suggest that genetic differences among full sib families were expressed while the plants were small, whereas resource limitation within pots imposed a more-or-less fixed ceiling on the total production of a pot when the plants were fully grown.

Production. The correlation between our two measures of production, total height and dry weight, is statistically significant but not strong ($r=0.57$, $n=88$, $p < 0.001$). Neither character is correlated with genetic diversity, a result corroborated by the analyses of variance (Table 2).

There is some indication that the final height of the progeny of inbred parents is less than that of the progeny of outcrossed parents (Table 2). This difference was also observed at the first measuring date, but subsequent relative growth rates were not significantly different. Thus, it seems that our inbred plants produced lower quality seed than our outcrossed plants; however, because parental type and parental environment were confounded, this result cannot be given an unequivocal interpretation.

Table 1. The mean range within pots for 16 phenotypic characters and statistical tests for differences among treatments.

Character	Mean range				F-ratio					Spearman correlation
	Inbred Sibs (n=18)	Outcrossed Sibs (25)	Outcrossed Nonsibs (25)	Inbred Nonsibs (20)	Model (df=24)	Block (21)	Rel (1)	Rel* Out (1)	Out (1)	(n=88)
Day 39										
Height (cm)	1.4	1.6	1.6	2.2	1.0	1.0	1.0	0.7	0.4	0.21+
Diameter (mm)	0.7	0.9	0.9	1.1	1.2	1.2	1.8	2.2	0.2	0.12
Day 75										
Height (cm)	4.8	6.0	6.4	11.4	1.2	1.0	3.7+	3.0+	0.8	0.23*
Diameter (mm)	0.9	1.3	1.2	2.6	1.0	0.6	1.2	7.9**	3.2+	0.19+
Day 116										
Height (cm)	3.8	6.2	8.8	16.3	1.8*	1.3	11.4**	4.4*	0.4	0.36***
Days 153-169 (harvest)										
Height (cm)	4.5	6.7	8.5	15.7	1.5+	1.2	7.6**	3.5+	0.8	0.31**
Internode 1 (mm)	8.7	6.7	11.9	12.0	1.1	1.0	4.7*	0.2	0.6	0.19+
Internode 2 (mm)	8.6	7.4	11.9	13.9	1.4	1.3	6.1*	0.0	0.5	0.20+
Internode 3 (mm)	11.4	11.9	14.6	21.5	0.8	0.7	3.8+	1.0	0.8	0.25*
No. of nodes	1.6	1.3	1.6	2.8	1.2	1.1	2.6	1.0	2.6	0.16
Diameter, min (mm)	0.8	0.7	0.8	1.8	1.1	1.0	2.0	1.6	4.3*	0.16
Diameter, max (mm)	1.0	1.2	0.9	1.8	1.0	1.0	0.3	2.0	1.7	0.13
Nodiness (mm)	0.6	0.7	0.5	0.7	1.4	1.5	0.2	2.5	0.2	0.08
Dry weight (g)	0.8	0.9	0.7	1.8	0.9	0.6	1.7	3.6+	4.1*	0.19+
No. of spider mites	0.11	0.09	0.06	0.12	3.1***	3.3***	0.3	0.7	7.9**	-0.02
No. of immature whiteflies	0.17	0.15	0.15	0.18	1.1	1.2	0.0	0.1	0.4	0.03

+ - $0.1 > p > 0.05$; * - $0.05 > p > 0.01$; ** - $0.01 > p > 0.001$; *** - $p < 0.001$; all two-tailed

All statistics calculated using square-root or log transformed ranges; means were then back-transformed. For spider mite and whitefly infestation, ranges calculated on square-root transformed data. For all ANOVA's, denominator df=63.

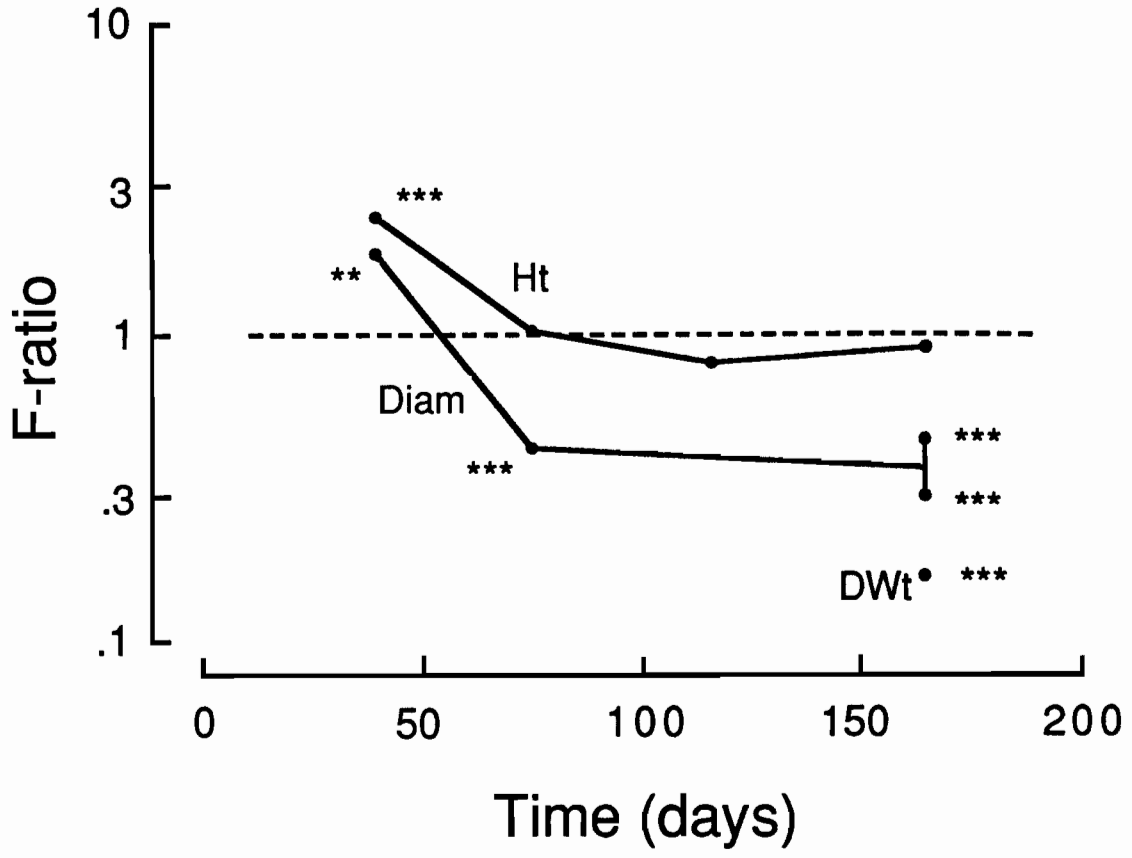

Figure 2. Changes in the relative variance of plant size within and among pots through the growing season. F-ratios calculated from single classification analyses of variance. Time measured from date of first germination. ** - $0.01 > p > 0.001$; *** - $p < 0.001$.

Table 2. The mean total production and infestation within pots and statistical tests for differences among treatments.

Character	Mean range				F-ratio					Spearman correlation
	Inbred Sibs (n=18)	Outcrossed Sibs (25)	Outcrossed Nonsibs (25)	Inbred Nonsibs (20)	Model (df=24)	Block (21)	Rel (1)	Rel* Out (1)	Out (1)	(n=88)
Final production										
Height (cm)	139	154	149	142	1.1	1.0	0.0	0.4	5.1*	-0.00
Dry weight (g)	6.2	6.9	6.5	6.7	1.4	1.3	0.0	4.0*	1.2	0.01
Components of production										
Height, day 39 (cm)	16.2	18.2	18.2	14.7	1.1	0.9	0.2	0.4	7.2**	-0.13
Residual height (cm)	-5.3	2.4	-1.8	2.4	1.3	1.3	0.1	2.0	0.0	0.12
Residual dry weight (g)	-0.25	0.10	-0.29	0.46	1.1	1.0	0.3	4.2*	2.2	0.11
Infestation										
Spider mites/leaf	0.051	0.063	0.031	0.064	4.1***	4.4***	0.5	3.8+	4.4*	0.02
Immat. whiteflies/leaf	0.21	0.23	0.24	0.26	4.2***	4.8***	0.1	0.2	0.0	0.16
Whitefly adults/pot	18.5	18.8	18.2	12.1	5.9***	6.6***	3.2+	2.4	0.6	-0.06

Infestation. The mean number of spider mites and immature whiteflies per leaf were 0.043 (0.032-0.055, 95% CL) and 0.22 (0.18-0.26) respectively; the mean number of whitefly adults collected per pot was 16 (13-22). Nested analysis of variance indicates that there are highly significant differences among pots within blocks for the mean number of both spider mites and immature whiteflies per leaf ($F_{66,88}$=2.1 and 1.9 respectively, $p \leq 0.002$). Further, the mean number of immature whiteflies per leaf correlates well with the number of whitefly adults across pots ($r=0.55$, $n=88$, $p < 0.001$). Together, these analyses suggest that our measures of pest infestation are reliable.

The number of spider mites is not correlated with the number of immature whiteflies ($r=-0.08$, $p > 0.4$) and only weakly with the number of whitefly adults ($r=-0.21$, $p=0.05$). None of the three measures of infestation was correlated with either total height or total dry weight (for all, $-0.02 \leq r \leq 0.12$, $p \geq 0.2$).

There is no indication of a relationship between genetic diversity of plants in a pot and any measure of infestation, either by correlation or ANOVA (Table 2). There is some slight tendency for more spider mites to be found on inbred plants, but again this result is difficult to interpret.

At a slightly larger spatial scale, we calculated the genetic variance of whole blocks using the identity of grandparents contributing to the block and Simpson's index of diversity ($D = 1-\sum f^2$). In theory this index can range from 0 to 1; however, in our experimental design it ranged only from 0.69 to 0.88. This measure of genetic diversity did not correlate with the number of spider mites, immature whiteflies or whitefly adults ($r=0.18$, -0.23, -0.02 respectively, $n=22$, $p \geq 0.3$).

DISCUSSION

The hypothesis under examination asserts that similar genotypes depress each other's fitness more than dissimilar genotypes; such a proposal figures prominently in several models for the evolution of sexual reproduction (Bell 1982, 1985; Price & Waser 1982; Tooby 1982; Case & Taper 1986). Two plausible mechanisms by which such an effect might occur include greater overlap of resource requirements and the more rapid

spread of parasites. Comparisons of genetically diverse and genetically uniform populations provide direct tests of the hypothesis.

The simplest and most general form of the hypothesis predicts an advantage to genetic diversity large enough to pay the two-fold cost of sex more-or-less regardless of the genotypes and environments concerned. The most extensive relevant data base is that on agronomic trials of crop mixtures and monocultures. In general, mixtures of crops and cultivars tend to support lower levels of both pathogens (Burdon 1987:47) and insect herbivores (Risch et al. 1983). However, the effect on yield seems to be minimal. A survey of 161 comparisons in 7 crops between monocultures and equal binary mixtures of cultivars revealed a consistent but very small tendency for mixtures to have greater seed yield than the mean of the component monocultures (mean advantage 1.8%, 95% C.L. 1.1-2.5%; G. Bell, unpublished MS; see also Trenbath 1974). Experiments involving more diverse mixtures, with about 10 varieties or cultivars, lead to similar conclusions (e.g. Clay & Allard 1969; Walker & Fehr 1978). The hypothesis in its simplest and most general form seems unlikely to be correct.

From the standpoint of a population biologist, the genotypes being mixed in these agronomic trials are somewhat arbitrary -- they have neither evolved together nor evolved in a genetically heterogeneous environment. Experiments in which mixtures of genotypes which have evolved together for 5-20 generations are compared to arbitrary mixtures have led to some suggestively large differences (Allard 1961; Allard & Adams 1969; Shorter & Frey 1979). In our experiment we went even further and derived all our subjects from a single natural population of I. capensis. We manipulated genetic variance in two ways, by comparing progeny of inbred and outcrossed parents and by planting sibs or nonsibs together. Despite evidence of resource limitation, there was no effect of genetic variance on productivity, nor was there an effect on infestation by spider mites and whiteflies. This latter result is perhaps not too surprising given that there was no evidence of genetic variance for susceptibility in the population, perhaps reflecting the fact that common greenhouse pests are likely to be extreme generalists. We are not aware of any other such study of infestation rates, but authors of more-or-less analogous

experiments report a similar absence of effect of genetic diversity on production (Table 3). The hypothesis is constrained still further.

Nevertheless, there remains an obvious out -- it will be noted that while our experimental material and that of the other studies in Table 3 was derived from an evolved mixture, the actual comparison of mixtures and monocultures was made in a greenhouse or experimental garden, not the environment in which the mixture evolved. It is conceivable that only in this environment will evolved mixtures markedly outperform monocultures. A very few studies have looked at the effect of neighbour relatedness on plant growth and infestation back in the parental source population -- these are the studies of Anthoxanthium odoratum in a mown field on the Duke University campus (Antonovics & Ellstrand 1984; Schmitt & Antonovics 1986; Kelley 1989a). In the only study of pest infestation, Schmitt & Antonovics (1986) report no significant difference in the number of aphids per plant between individuals flanked by 4 sibs and those flanked by 4 nonsibs. Effects on fitness components have been ambiguous. Antonovics & Ellstrand (1984) report suggestively large mean differences in performance between tillers flanked by clones or nonclones (production ratios of 1.5 and 2.2), but with considerable variance and skew in the data such that confidence limits were wide and statistical significances test-dependent. Schmitt & Antonovics (1986) compared survival rates of individuals flanked by either 4 sibs or 4 nonsibs -- there was no difference for aphid-free plants ($p > 0.8$), but among aphid infested plants those flanked by nonsibs survived about 30% better ($0.05 > p > 0.02$). There was no difference in the probability of reproducing ($p > 0.9$). Finally, as part of a larger experiment, Kelley (1989a) compared the output of patches of 8 tillers from either 2 or 4 parents. There was no significant difference for either sexually derived tillers or asexually derived tillers.

To conclude, the weight of evidence indicates that mixtures do not significantly outperform monocultures when either arbitrary mixtures of genotypes are used or when evolved mixtures are used in an arbitrary environment. The behaviour of evolved mixtures in the environment in which they evolved remains largely unknown.

Table 3. Survey of greenhouse and garden experiments comparing plant production in genetically diverse and uniform stands using genotypes derived from a natural population. Production ratio calculated as ratio of production in diverse treatment to production in uniform treatment; for experiments with more than two treatment levels, only the most diverse and the most uniform were used.

Species Design (Reference)	Measure of production	Subjects	Production ratio (Diverse/Uniform)
Impatiens capensis			
Greenhouse - 4 seeds in a pot from either 1 or 2 parents (1)	Dry wt	CH plants	0.9
		CL plants	1.1
Greenhouse - seeds flanked by either 2 sibs or 2 nonsibs (2)	Dry wt	CH plants	1.1
		CL plants	1.0
Garden - seeds flanked by either 2 sibs or 2 nonsibs (2)	Dry wt	CH plants	1.4
		CL plants	0.9
This study	Dry wt		1.1
	Height		1.0
Anthoxanthum odoratum			
Greenhouse - 4 tillers in a pot from either 1 or 4 parents (3)	No. of inflorescences (2 yrs)	Sexual tillers	0.8
		Asexual tillers	1.2
Greenhouse - 6 tillers in a pot from either 1 or 6 parents (3)	No. of inflorescneces (2 yrs)	Sexual tillers	1.3
		Asexual tillers	1.0
Greenhouse - 8 tillers in a pot from either 2 or 4 parents (3)	No. of inflorescences (2 yrs)	Sexual tillers	1.1
		Asexual tillers	0.7

Phytolacca americana		
Greenhouse - 3-5 seeds/pot either sibs or nonsibs (4)	Height	1.0
Greenhouse - 7-10 seeds/pot either sibs or nonsibs (4)	Height	0.9
Abutilon theophrasti		
Greenhouse - 3-5 seeds/pot either sibs or nonsibs (4)	Height	0.9
Solanum mauritanianum		
Greenhouse - 4-8 seeds/pot either sibs or nonsibs (4)	Height	1.0
Lycopersicon lycopersicon		
Greenhouse - 4-8 seeds/pot either sibs or nonsibs (4)	(1) Height	1.0
	(2) Height	1.0
Plantago lanceolata		
Greenhouse - 3 seedlings/pot either full sibs or nonsibs (5)	Total dry wt.	? (ns)
	No. vegetative shoots	? (ns)
	Vegetative dry weight	? (ns)
	Probability of flowering	0.67 (0.005>p>0.001)
	No. infructescences	< 1 (0.05>p>0.025)
	Seeds/fruit	? (ns)
	Length of inflorescence	? (ns)
	Reproductive dry wt.	? (ns)

Notes: Unless otherwise indicated, differences are not statistically significant. Some values are estimated from published figures.

References: 1- Schmitt & Ehrhardt (1987); 2- McCall et al. (1989); 3- Kelley (1989b); 4- Willson et al. (1987); 5- Tonsor (1989).

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CHAPTER EIGHT

Modelling the quantitative genetics of fitness

ABSTRACT

The infinite alleles model often used to study stabilizing selection is transformed by slight modifications into a quantitative genetic model of fitness. Negative epistasis for fitness is likely to be a condition for equilibrium and selection of all kinds tends to produce negative correlations between loci, contrary to the conventional oligogenic model. Sex and recombination are found to increase population mean fitness. An expression for the equilibrium genetic variance of fitness is derived using Fisher's Fundamental Theorem of Natural Selection; a value of 0.01 based on the mutation pressure in Drosophila is probably conservative. This value may be sufficient to support 'good genes' mate choice, but will be all but impossible to measure in the field.

* * *

Many continuous phenotypic characters are thought to be under stabilizing natural selection, and the evolution of such characters has been extensively modelled by theoretical quantitative geneticists (e.g. Latter 1960, 1970; Kimura 1965; Lande 1976; Bulmer 1980). In these models the fitness of an individual is a decreasing function of the deviation of its phenotype from some optimal value; the paradigmatic character is abdominal bristle number. Curiously, rather less attention has focussed on the evolution of fitness itself. Fitness differs from the characters considered previously in two important respects: first, mutations and changes in the environment are biased, tending to decrease mean fitness; second, natural selection is directional, tending to increase mean fitness. In this paper I describe a slight modification of the infinite alleles model used in the study of stabilizing selection which incorporates these peculiarities of fitness. The model is meant to capture most of what (little) we know about fitness. I then analyse two properties of the model: 1) the conditions for equilibrium; and 2) the consequences of recombination. Because the character being modelled is fitness, the results are important for our understanding of population genetics in general and the evolution of recombination in particular. I also develop expressions for the equilibrium genetic variance for fitness and the advantage of female choice. These do not depend on any particular model of fitness, utilizing instead Fisher's Fundamental Theorem of Natural Selection.

THE MODEL POPULATION

Individuals are haploid with n loci and generations are discrete. Each allele at each locus has a 'genotypic value', G; values for any one locus are continuously distributed, and so there are an infinite number of possible alleles. The genotypic value of an individual is the sum of all its alleles, and this determines its expected number of offspring according to some monotonically increasing fitness function. The net effect of mutation and changes in the environment is to reduce the mean genotypic value (and mean fitness); the effect of selection is to increase it.

This 'genotypic value' has been introduced for the convenience of the theory, not because it is readily observable or of primary importance (it is neither). It might be thought of as some abstract phenotypic character, like vigour. Using the language of classical genetics, it is the number of wild type alleles (or, equivalently, the opposite of the number of mutations). The important point about genotypic values is that they are additive across loci, and the fitness of an individual is a monotonically increasing function only of the sum of its alleles. Put another way, genotypic values are the fitness effects of alleles transformed such that they combine additively. The difference between two alleles may be large or small, and will change between generations due to mutation and/or environmental change. On the fitness scale, the difference will also usually depend on the alleles at other loci. Only if the fitness of an individual is given by W=expG is there no deviation from multiplicative interactions; all other fitness functions lead to epistasis (Felsenstein 1965). Note, though, that changes in the alleles at other loci cannot cause an out-and-out reversal of which allele is more fit. I am assuming that such extreme interactions are of negligible importance among naturally occurring polymorphisms.

To model the effects of mutation and changes in the environment, both of which tend to degrade the fit between genotype and environment produced by natural selection, all genotypic values are changed between generations. More precisely, in a population with n loci, the genotypic value of the ith allele in the jth individual in generation t+1 is:

$$G_{i,j,t+1} = G_{i,j,t} - (\mu/n + \varepsilon_{i,j}) \qquad (1)$$

where μ/n is the mean change, $\mu>0$, and $\varepsilon_{i,j}$ is a random variate with mean zero and variance σ_m^2/n which is assumed to be independent of the genotypic value in generation t. (In the language of classical genetics, the mutation rate is independent of the mutational load.) Furthermore, changes at different loci are assumed to be independent, so each generation the mean genotypic value of individuals decreases by μ and the variance among individuals increases by σ_m^2. Equation (1) will be referred to as the degradation function.

CONDITIONS FOR EQUILIBRIUM

The evolution of the model population is wholly determined by two forces, mutation/environmental deterioration and selection, one tending to decrease mean fitness, the other to increase it. I now ask what combinations of degradation and fitness functions result in the population evolving to some sort of stable state. Such equilibria are readily derived from the conventional single locus treatments of mutation-selection balance (Crow & Kimura 1970: 258-262). In these models the locus being considered affects some easily distinguished Mendelian character, there is a clearly defined 'wild type', and it is reasonable to assume that the deleterious effects of a mutation are very large compared to the mutation rate. Such models are not appropriate for modelling fitness. Here I investigate the conditions for equilibrium in a population with an infinite number of possible genotypes; as the population is asexual, I need only consider a single locus.

In order for the population to be at a stable equilibrium, the expected change in G due to selection must equal the expected change due to mutation and environmental deterioration. Furthermore, if G should happen to deviate from the equilibrium value, then there must be a tendency for it to return to this value. This tendency to return must derive from the degradation function and/or the fitness function. By assumption, the degradation rate is independent of the genotypic value; thus, the presence or absence of an equilibrium will depend on the fitness function. Specifically, if the function relating genotypic value to the logarithm of fitness is f(G) (i.e. $\ln W=f(G)$, or $W=\exp(f(G))$) then a stable equilibrium between mutation and selection is reached only if the second derivative of f(G) is negative ($f''(G) < 0$). I have not been able to prove this conjecture

analytically, but it is supported by both intuitive argument and computer simulation.

The intuitive argument goes as follows. We have seen that in order for there to be an equilibrium point, the population must return to it after a perturbation, and that this tendency to return must be supplied by selection. That is, if G should happen to drift below the equilibrium value, then the expected change in G due to selection (ΔG_s) must increase; if G should happen to drift above the equilibrium, then ΔG_s must decrease. There must be a negative correlation between mean G and ΔG_s. The latter depends on the intensity of selection, which in turn depends on the CV^2 of fitness, or, equivalently, the variance of the logarithm of fitness. For a given variance in G, the variance in lnW depends on the slope of the function relating lnW to G - the steeper the slope, the higher the variance on lnW. To restate the condition for equilibrium then, there must be a negative correlation between G and the slope of the function relating lnW to G. This occurs precisely when the second derivative of the function is negative. Other fitness functions do not lead to a stable equilibrium. If the second derivative is zero, then G will either increase or decrease at a constant rate, depending on the relative magnitudes of the changes due to degradation and selection; if they should exactly cancel out, then the population would be at a neutral equilibrium. If the second derivative is positive, then there will be an equilibrium point, but it will be unstable, a decrease in mean G resulting in less intense selection, and an increase in mean G resulting in more intense selection. Under such conditions, the population mean fitness would either increase or decrease at an accelerating rate, until either the fitness function changed or the population went extinct.

This intuitive argument is supported by Monte Carlo analysis. The computer program simulated asexual populations of N=500 individuals subject to a degradation function of the form of equation (1) and a variety of fitness functions. The simulations were run for 1000 generations, with two or more initial genotypic values per fitness function, and with 10 replicates of each. Fitness functions with negative, zero, and positive second derivatives on a logarithmic scale were used; they are shown in Figure 1 on an arithmetic scale. Additive and Gaussian fitness functions have negative second derivatives and result in convergence to a stable equilibrium (Fig. 2a,b); though not differentiable, threshold selection also

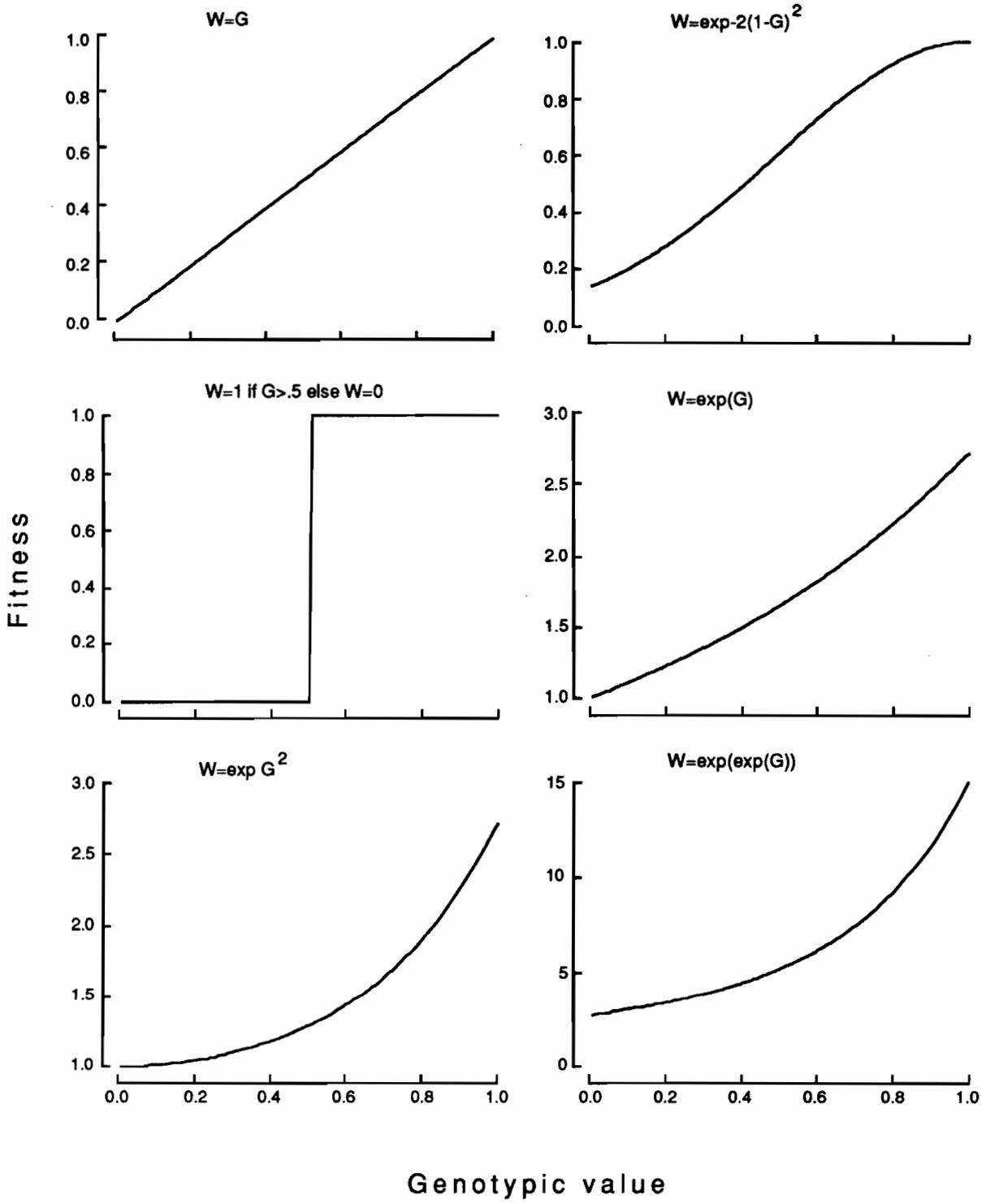

Figure 1. Six fitness functions used in Monte Carlo simulations. a) additive; b) gaussian; c) threshold; d) multiplicative; e) squared; and f) double exponential.

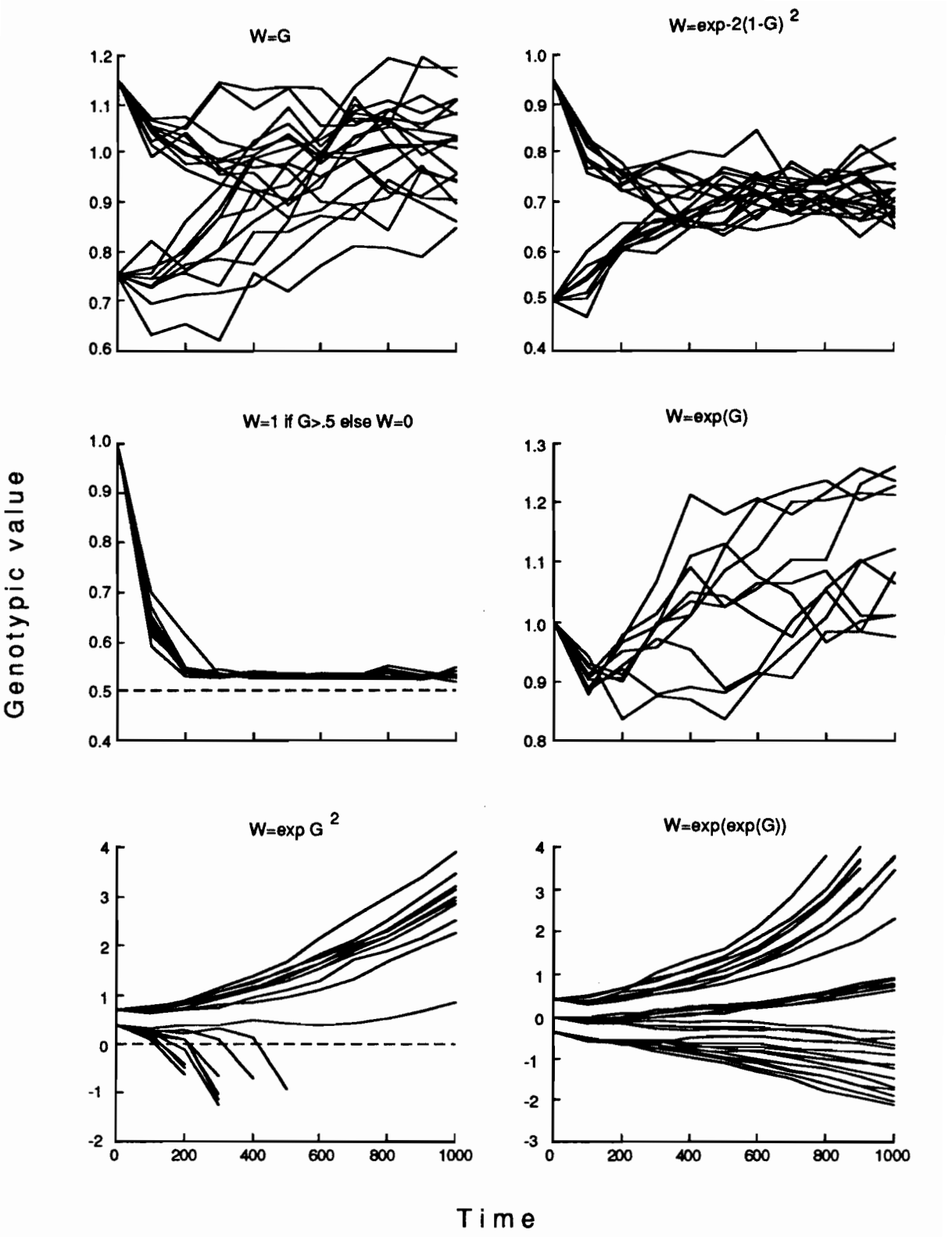

Figure 2. The evolution of genotypic value in Monte Carlo simulations using six different fitness functions. For each starting genotypic value in each graph there are 10 replicate runs. For the degradation function (equation 1), n=1, μ=0.004, and σ_m^2=0.0001. Populations started each run with zero genetic variance.

leads to a stable equilibrium as long as the population starts above the threshold (Fig. 2c). Multiplicative selection, with zero second derivative, leads, with the parameter values used, to a constant increase in G (Fig. 2d); other values would result in a constant decrease. Finally, fitness functions with positive second derivative show divergence from an equilibrium point at an ever-increasing rate (Fig. 2e,f).

To conclude, then, if the degradation of genotypic values due to mutation and environmental changes is independent of the genotypic value, then the logarithm of fitness must fall off faster than linearly with the genotypic value in order for the population to reach a stable equilibrium. These results differ from those of the classical single locus models of mutation-selection balance. These latter readily lead to an equilibrium both because the number of mutants created falls off as the wild type becomes less frequent and because the change in frequencies due to selection falls off as an allele becomes either very rare or very common. Such models do not seem appropriate when modelling fitness itself. In particular, we cannot assume that there are a limited number of alleles or that the deleterious effects of mutation are large compared to the mutation rate, since the locus here is the entire genome. For these reason I have used an infinite alleles model.

If this model is an accurate description of natural populations, then the genetic variance observed in nature will show negative epistasis for fitness. This prediction can be tested with a variety of experimental designs. In haploids, for example, the theory predicts a negative correlation between the difference between offspring and parental mean ln fitness and the standard deviation of parental ln fitness (Fig. 3). Secondly, if a population is allowed to propagate asexually for many generations, thus building up negative correlations between loci (see next section), then the theory predicts that sexually produced offspring will have lower mean ln fitness than those asexually produced. Finally, if one removes the effect of selection, allowing mutation pressure to drive evolution, then ln fitness should fall off at an ever-increasing rate. Such a pattern has been observed in Drosophila melanogaster by Mukai (1969), and seems to be a general property of evolution in isolate cultures (Bell 1989:34-35). Note, though, that these results might also be due to a degeneration of the DNA copying and repair systems during the course of the experiments.

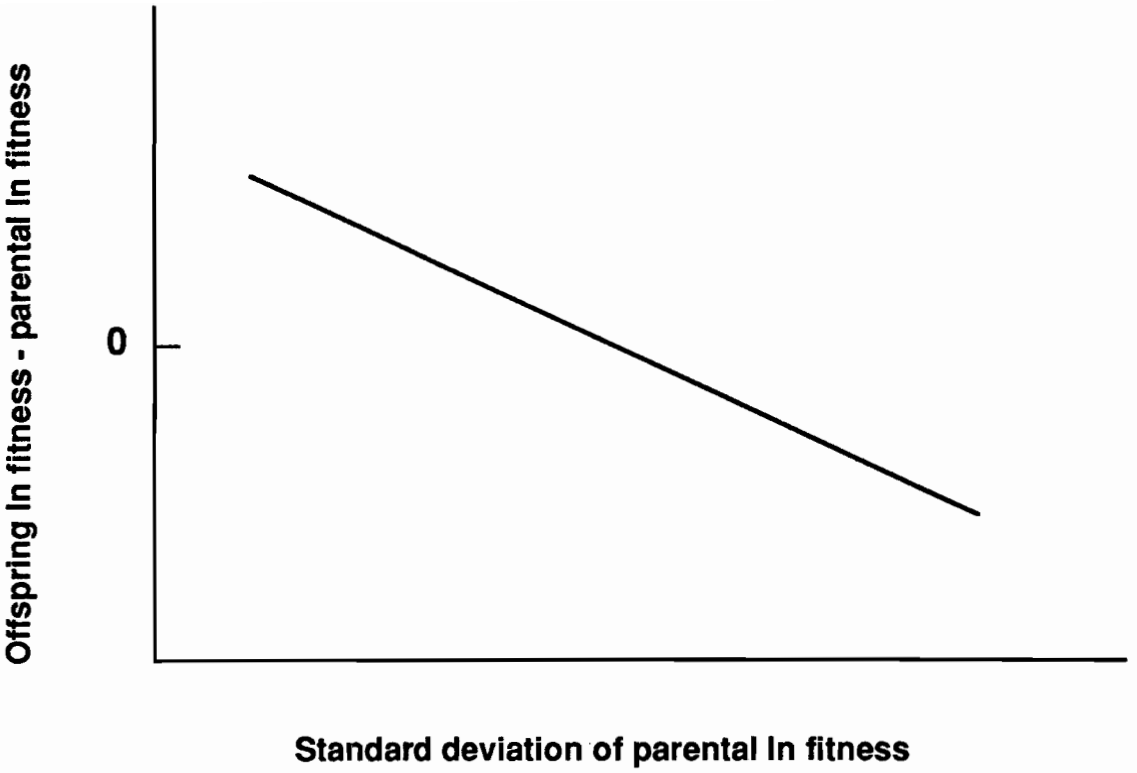

Figure 3. If there is negative epistasis for fitness as predicted by the model then the difference between offspring and parental mean ln fitness should decrease for more dissimilar parents.

CORRELATIONS AMONG LOCI AND THE CONSEQUENCES OF RECOMBINATION

To now I have considered an asexual population; I now wish to investigate the population genetic consequences of sex and recombination, which will require keeping track of at least two loci. The primary effect of recombination in a panmictic population is to break down correlations between alleles at different loci; these latter may be produced by drift or, more importantly, by natural selection. The effect of this randomization of allele combinations on genetic variances, rates of evolution, and population mean fitnesses will depend on whether the genetic correlations being broken down are positive or negative. If positive, then recombination will tend to reduce the genetic variance among individuals, and thus the rate of evolution and the mean fitness at equilibrium; if negative, then the opposite. Thus, I shall start by considering the effect of selection on the association of alleles at different loci.

Correlations among loci. According to the conventional oligogenic model, the sign of the genetic correlation produced by directional selection is the same as the sign of epistasis (Felsenstein 1965). Imagine a population initially in linkage equilibrium. If alleles at different loci combine multiplicatively to determine fitness (no epistasis, the second derivative of ln fitness on genotypic value being zero), then the population will remain in linkage equilibrium. If fitness increases faster than multiplicatively (positive epistasis; positive second derivative), then positive correlations between loci are produced. Finally, if fitness increases slower than multiplicatively (or falls off faster than multiplicatively; negative epistasis; negative second derivative), then negative correlations between loci are produced. This theoretical result seems to be generally accepted and has been widely used in discussions on the consequences of sex and recombination (e.g. Maynard Smith 1968, 1978, 1988; Thompson 1976; Felsenstein 1988; Charlesworth 1989).

To test whether these ideas apply to an infinite alleles model, the above simulations were changed slightly so that evolution at two loci, A and B, was followed. Each generation the genotypic values at A and B decreased as follows:

$$A_{i,t+1}=A_{i,t} - (\mu/2 + \varepsilon_{Ai})$$

and

$$B_{i,t+1}=B_{i,t} - (\mu/2 + \varepsilon_{Bi})$$

where μ=0.004 and ε_{Ai} and ε_{Bi} are independent random variates with mean zero and variance $\sigma_m^2/2$ = 0.00005, as before. Note that since the population is still asexual, its behaviour is indistinguishable from that of the one-locus model; the only difference is in what variables are recorded - in particular, I followed the covariance of genotypic values between the two loci. Note also that the degradation function does not change the covariance between loci, and drift tends only to reduce the absolute value of the covariance to zero, so any covariance generated in the model must be due to selection.

Results for the six fitness functions considered previously are shown in Figure 4. Each graph shows means ± twice the standard error based on 30 replicate runs, and in each the top line is the variance in total genotypic value among individuals and the bottom line is twice the covariance of values at the two loci. For all fitness functions, including those with positive epistasis, selection produces negative correlations between loci. The conventional results from the oligogenic model do not apply to the infinite alleles model.

An intuitive explanation of the results in Fig. 4 may be given as follows. The covariance between A and B can be represented as a function of the variance in their sum and the variance in their difference:

$$\text{cov}(A,B)=[\text{var}(A+B)-\text{var}(A-B)]/4 \quad .$$

Thus, the sign of covariance depends on the relative magnitude of the variance in the sum and in the difference. Degeneration and drift affect both of these equally. Directional selection tends to reduce variance, and will reduce the variance of A+B more than that of A-B because the former is more closely related to fitness. Put another way, selection is more intense on A+B than on A-B (which in fact is neutral in this model), and so negative correlations appear, regardless of the form of epistasis.

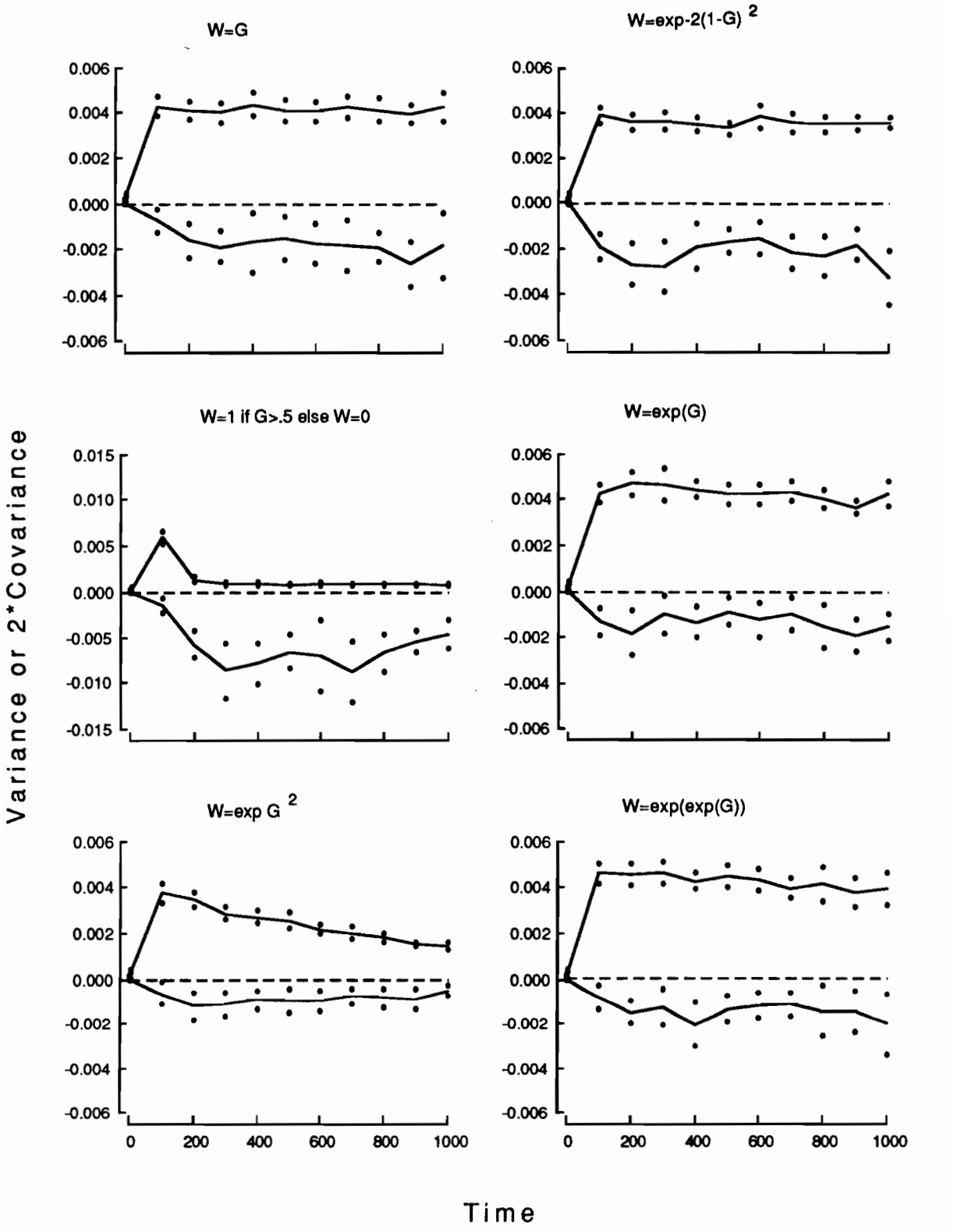

Figure 4. The evolution of variances and covariances in a two locus population. Initial genotypic values were 1.15 (Additive), 0.95 (Gaussian), 1 (Threshold), 1 (Multiplicative), 0.7 (Squared), and 0 (Double exponential).

The result from the oligogenic model derives from the fact that the variance of the difference is not the same for all phenotypes (Fig. 5) and so selection can change the variance in differences as much as or more than the variance in sums. This in turn is a consequence of there being a limited number of alleles with pre-determined fitness effects. For continuous characters like fitness, the infinite alleles model seems preferable.

A slightly more quantitative treatment goes as follows. Consider a population with n equally variable loci and in which the genotypic value of an individual is equal to the sum of its n alleles. By definition, the regression of values at one of these loci, say A_i, on the total genotypic value has slope 1/n, regardless of the covariance among loci. Thus, if the change in the variance of genotypic value due to selection is ΔV_{Gs}, then the change in the variance of A_i due to selection is $(1/n)^2$ times this:

$$\Delta V_{Ais}=(1/n)^2\Delta V_{Gs} \quad .$$

Summing these changes over the n loci, we get

$$\Sigma\Delta V_{Ais}=(1/n)\Delta V_{Gs} \quad .$$

The change in the covariance summed over all n(n-1) pairs of loci is thus, by subtraction,

$$\Sigma\Delta\mathrm{cov}(A_i,A_j)=(1-1/n)\Delta V_{Gs} \quad .$$

For any specific pair of loci, the change in covariance is

$$\Delta\mathrm{cov}(A_i,A_j)=(1/n)^2\Delta V_{Gs} \quad ,$$

the same as the change in the variance of a locus.

Thus the change in the covariance due to selection has the same sign as the change in the variance of genotypic value. Since selection on G is directional and thus reduces the variance of G, it follows that selection also acts to produce negative covariances between loci.

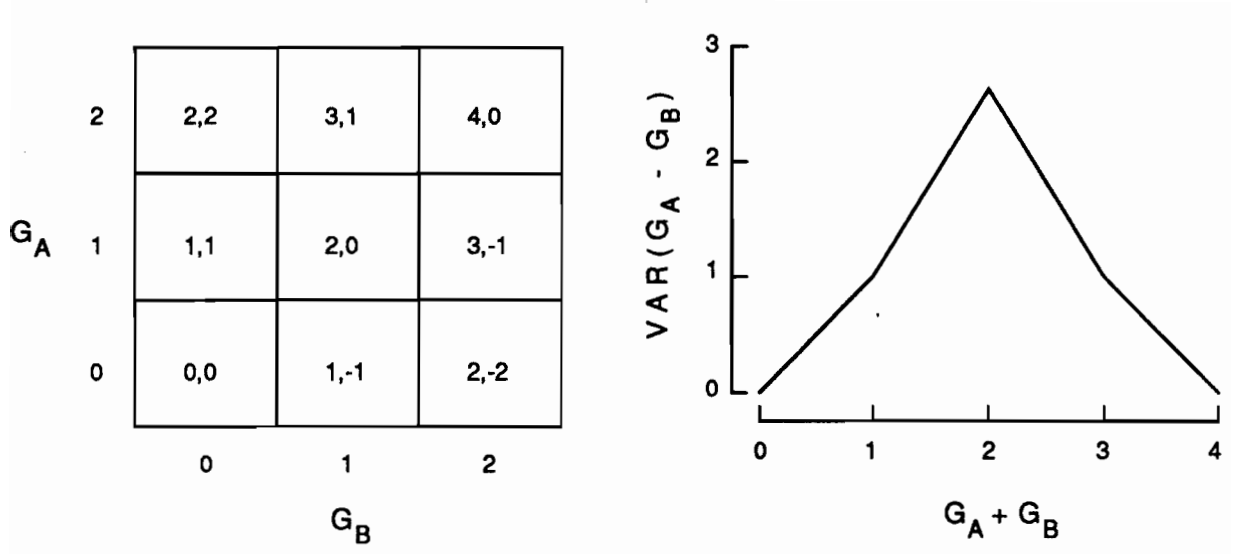

Figure 5. The conventional oligogenic model. There are three possible alleles (0, 1, and 2) at each of two loci (A and B). Genotypic values combine additively to produce the phenotype. Left: The values in each cell are the sum ($G_A + G_B$) and the difference ($G_A - G_B$) of genotypic values for each of the nine possible phenotypes. Right: Only four phenotypes are possible, and for each the variance in the difference across genotypes is shown. Note that the variance in difference is not the same for all 4 phenotypes. This means that directional selection on the phenotype can, as a correlated response, change the population-wide variance in differences. Most importantly, it can reduce the variance in differences more than the variance in sums, producing positive covariance between loci. In an infinite alleles model the variance in differences is equal for all phenotypes, and so this correlated response to selection cannot occur.

These equations also allow one to see what happens as one considers more and more loci. The most important consequence is that a larger and larger fraction of the change in the variance of G due to selection goes into negative correlations between loci and less and less into changes at the individual loci. At the limit of infinite loci, all of the change goes into negative correlations (Bulmer 1980 discusses this model in greater detail). As well, since the correlations between all pairs of loci are negative, they are mathematically constrained to being small in magnitude. For n equally variable parts of the genome, all negatively correlated, the average correlation between them must be $|r| \leq 1/(n-1)$. Studies attempting to test the prediction that selection produces negative covariances for fitness by directly measuring the correlation between different parts of the genome (cf. Thoday & Gibson 1972) will want to look at substantial fractions thereof.

Consequences of sex and recombination. The conclusion of the previous section was that selection of all sorts is likely to lead to negative correlations for fitness among alleles at different loci. It follows that sex and recombination, which tend to randomize the association of alleles, will increase the genetic variance of fitness, and thus the response to selection and the mean genotypic value. This conclusion has been confirmed by simulating a sexual population with two loci and recombination rate 0.5 under exactly the same conditions as the asexual populations of Figure 4. Table 1 shows the mean genotypic value for sexual and asexual populations, and for all six fitness functions it is higher in the sexual population than in the asexual population.

The advantage of a sexual population over an asexual population will obviously depend in some way on the various parameters in the model. To understand the factors influencing the advantage of sex, it is important to understand the flow of variance, for which reference to Figure 6, adapted from Lande (1976), will be useful. The total variation in the population can be divided into the component expressed among individuals and that hidden within genomes due to negative correlations. If the population were in linkage equilibrium, then this second fraction would be zero. In our model the degradation function increases the expressed variance, but, because it

Table 1. Mean genotypic values in asexual and sexual populations for 6 fitness functions.

Fitness function	Genotypic value (mean ± s.e.)	
	Asexual	Sexual
W=G	1.034±0.0175	1.153±0.0244
W=exp-2(1-G)^2	0.728±0.0087	0.748±0.0099
W=1 if G>.5 else W=0	0.531±0.0012	0.560±0.0034
W=exp(G)	1.112±0.0266	1.375±0.0859
W=expG^2	2.990±0.0830	4.259±0.1341
W=exp(exp(G))	0.371±0.1544	1.268±0.3014

Note: Initial conditions as for Fig. 4. Values given for T=1000 generations, by which time populations under the first three fitness functions have reached equilibrium; those under the last three never reach equilibrium (Fig. 2). Sexual population has two loci with r=0.5. Asexual values based on 30 replicate runs; sexual values on 10 runs.

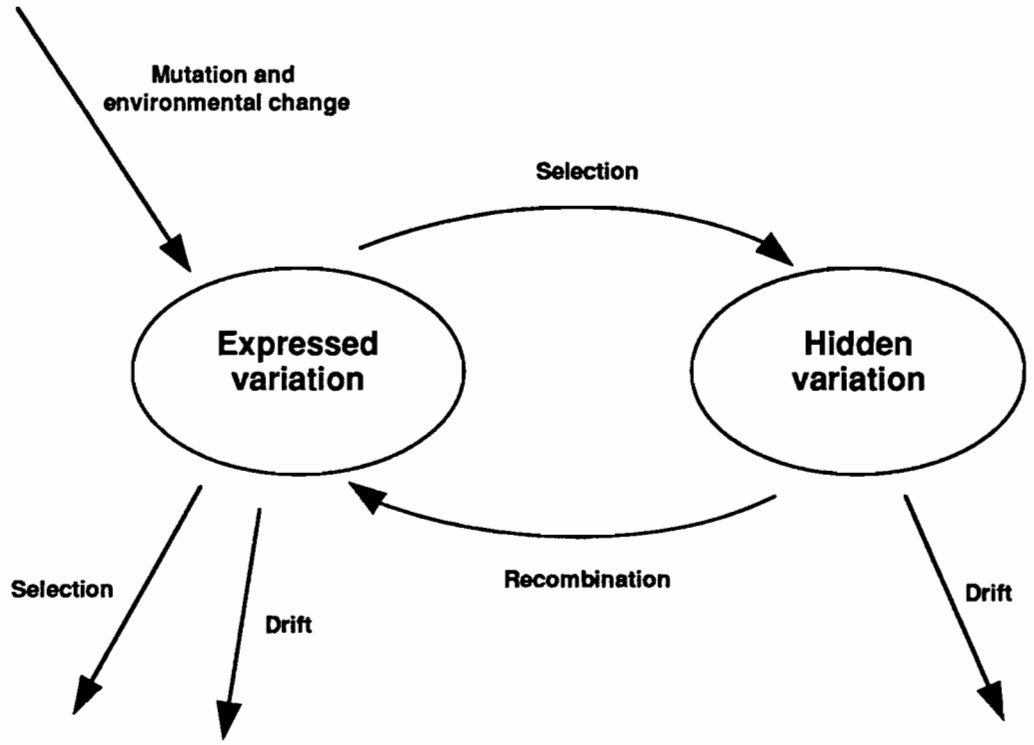

Figure 6. The flow of variance in the model population (adapted from Lande 1976).

occurs independently at different loci, it has no effect on the hidden variance. Selection reduces the expressed variance and increases the hidden variance. Recombination depletes the hidden variance, converting it back into expressed variance. Finally, drift reduces both the hidden and the expressed variance.

Consider two loci, A and B, between which selection produces negative covariance. Recombination will reduce the covariance by a factor of r and drift by a factor of 1/N. In an asexual population, or other population in which the effect of drift predominates (i.e. $1/N >> r$, or $rN << 1$), then the covariance produced by selection is lost to the population forever. However, in a sexual population in which the effect of recombination predominates (i.e. $rN >> 1$), then the hidden variance is recycled back into variance among individuals. In this case, recombination acts to counter the effects of selection and drift, and the result is a higher equilibrium variance among individuals on which selection may act.

This intuitive explanation allows one to understand how changing parameters in the model will affect the advantage of a sexual population. First, as noted, recombination functions to increase the expressed variance, and thus is most advantageous when this is scarce - in small populations (Fig. 7a). This finding is compatible with results from a number of computer simulation studies on rates of evolution in sexual and asexual populations (reviewed in Thompson 1976) and on the accumulation of deleterious mutations due to Muller's ratchet (Bell 1989).

Second, note that the exact value of the rate of recombination does not really matter, as long as it produces an effect much larger than that of drift (i.e. $r >> 1/N$). For example, in a population of 10,000 individuals, a recombination rate of 0.01 will have a very similar effect as one of 0.5; both will have a higher equilibrium variance than an asexual population ($r=0$). Much the same is observed in populations of 500 individuals (Fig. 7b). This conclusion is equivalent to Lande's (1976) claim that the equilibrium variance at mutation-selection balance does not depend on the rate of recombination between loci, as long as the effect of drift can be ignored.

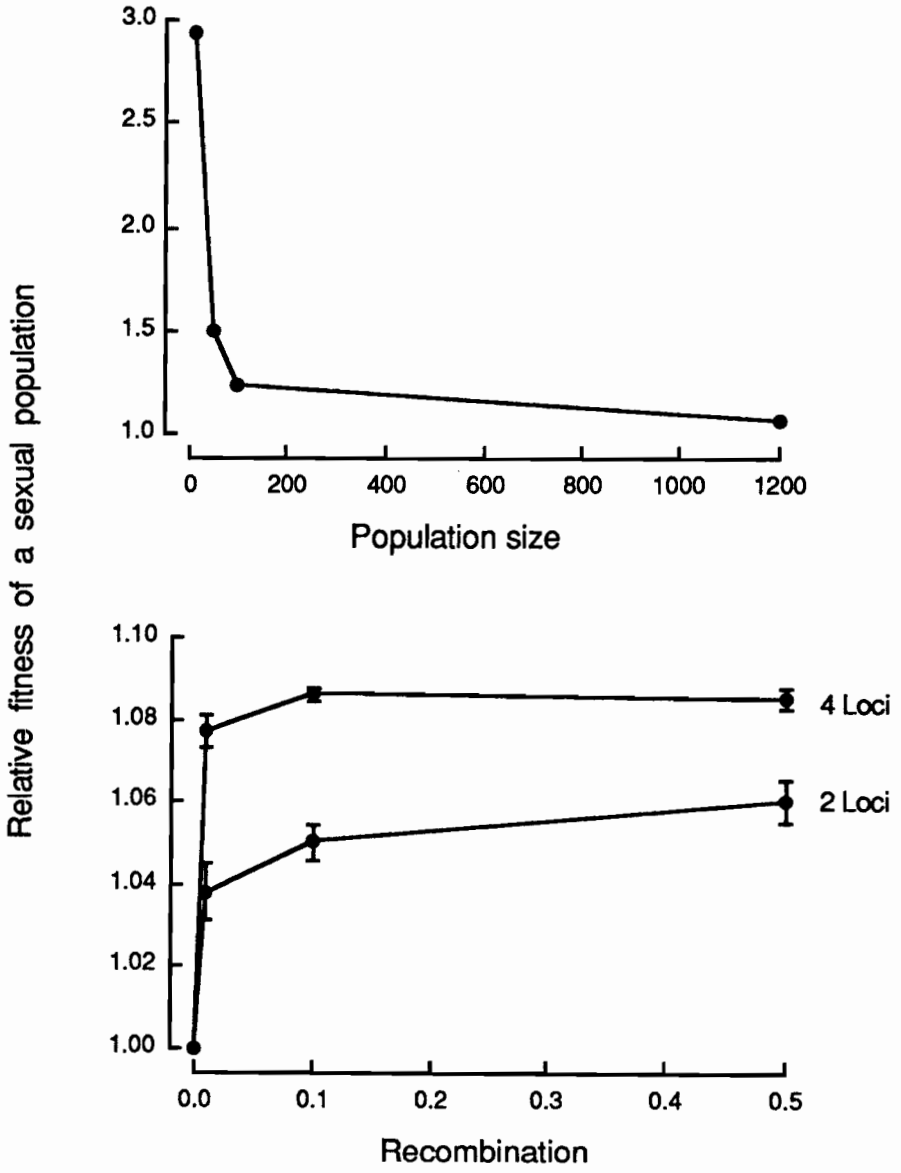

Figure 7. Top: The effect of population size on the population-level advantage of sex. Bottom: The effect of rate of recombination and number of loci on the advantage of sex. All values based on 10 sexual and 10 asexual runs; unless otherwise specified, the model parameters are: μ=0.004; σ_m^2=0.0001; N=500; n=2; r=0.5.

What does count much more than the precise rate of recombination between loci is the number of loci between which there is this minimal amount of recombination. As noted, for n loci, all but 1/n th of the reduction in expressed variance due to selection goes into negative correlations between loci, and is thus recyclable. The simulations demonstrate that the advantage of sex is greater in a 4 locus population than a 2 locus population (Fig. 7b). Lande (1976) similarly concludes that for fixed total input of mutational variance, the equilibrium expressed variance increases with the number of recombining loci.

According to this view, the population-level advantage of sex is not determined by the mean rate of recombination between loci, but rather by the number of loci between which there is a certain minimum amount of recombination. It follows that the number of chiasmata at meiosis is probably much more important in determining the effect of sex than the haploid number of chromosomes - adding a chromosome (or a localized cross-over) increases n by 1, but if the minimum recombination is, say, 0.01, then a crossover which forms randomly along the genome will increase n by 100.

The advantage of sex also responds to changes in the degradation function. With mean 0.004 and variance 0.0001, the advantage of sex is about 5%. Increasing the mean degradation rate to 0.007 increases the advantage of sex to about 50%. Leaving the mean at 0.004 and halving the variance to 0.00005 has an even larger effect, raising the advantage of sex to about 220% [Each of these values is based on 10 sexual and 10 asexual runs of 500 individuals with the gaussian fitness function. The sexual population has two loci with r=0.5.] Both increasing the mean degradation and decreasing the variance has the effect of making selection more intense, which in turn makes sex more advantageous. Note, though, that even under the conditions leading to a greater than two-fold advantage of sex, the intensity of selection was not unrealistically high. The average coefficient of variation of fitness in the sexual population was 0.180 ± 0.0063 (s.e.), which is fully compatible with what is known about the variance of fitness in natural populations (see below). These simulations indicate that the population-level advantage of sex can be high under quite reasonable assumptions, and suggest that it will be worthwhile to see if the forces described here can account for the spread of a modifier for sex and

recombination through a population. This will be a topic of future research.

THE GENETIC VARIANCE OF FITNESS AND THE ADVANTAGE OF FEMALE CHOICE

The genetic variance of fitness. Estimates of the genetic variance in fitness are of interest both in their own right and as they relate to 'good genes' theories of sexual selection. However, direct field estimates are exceedingly difficult to make and it is therefore useful to investigate alternative approaches. Charlesworth (1987) and Rice (1988) have derived an estimate for the expected equilibrium variance in fitness under mutation-selection balance using the data of Mukai et al. (1972) on the strength of mutation pressure in Drosophila melanogaster. However, their derivations are quite elaborate and involve quantities which can only be very tenuously estimated from the experimental data (e.g. per genome mutation rates, average homozygous and heterozygous fitness effects; see also Crow & Simmons 1983). Here I present an alternative derivation which has the advantages of being both simpler and using a more directly measurable quantity.

According to Fisher's Fundamental Theorem of Natural Selection, the relative change in mean fitness due to selection equals the square of the coefficient of variation of fitness (Crow & Kimura 1970: 209):

$$\Delta w/_{w} = CV^2_w$$

Thus we can use the relative change in fitness due to selection as an estimate of the CV^2 of fitness. A conservative estimate of this quantity can be calculated from the data of Mukai et al. (1972). Insofar as was possible, these investigators eliminated selection on and recombination of the second chromosome of Drosophila melanogaster, allowing only change due to mutation pressure. Periodically, larval viability was measured in the original selective environment. As expected, this measure of fitness declined over successive generations. From an original value of 1, fitness decayed with slope -0.00443 ± 0.00030 (s.e.) per generation, excluding lethals. The second chromosome of D. melanogaster contains about 2/5 of

the total DNA, so scaling up for the whole genome gives an estimate of about -0.01 per generation. If mutation is reducing mean fitness by 0.01 each generation, then at equilibrium selection must be increasing it by this amount, and, by the Fundamental Theorem, the CV^2 of fitness must also be about 0.01. This value is about twice that estimated by Charlesworth (1987) and three times that estimated by Rice (1988) and it is well within the range of observed additive genetic coefficients of variation for components of fitness (data summarized in Charlesworth 1987). Nevertheless, several factors suggest that it is still likely to be very conservative.

First, the measured strength of mutation pressure is likely to be an underestimate both because selection could not have been removed completely and because the effect was only measured on one component of fitness (larval viability).

Second, the effect of sex and recombination ought also to be considered. As seen above, sex will tend to increase the variance of mutational load. The effect of this on mean fitness depends on the shape of the function relating fitness to load. The best available evidence, also for the second chromosome of D. melanogaster, suggests that this function has a negative second derivative (Mukai 1969), and thus that an increase in the variance of load would lead to a decrease in mean fitness. This conclusion is supported by the observation that recombined second chromosomes of D. melanogaster have lower fitness than unrecombined chromosomes (Charlesworth & Charlesworth 1975; but cf. Kelley et al. 1988). These results suggest that at equilibrium selection must counteract the deleterious effects of sex and recombination as well as of mutation, and thus that the value derived above is an underestimate.

Finally, and most importantly, natural populations live in a more heterogenous world than fruit flies in vials. Changes in the environment through time and immigration from different environments will tend to decrease mean fitness and thus require a greater equilibrium change in fitness due to selection, and thus a greater equilibrium CV^2 of fitness.

These considerations suggest that the estimate of $CV^2_w=0.01$ derived above is likely to be conservative, conceivably by as much as an order of magnitude. How should it be interpreted? The answer depends on one's perspective. From the standpoint of sexual selection theory, a genetic standard deviation of 0.1 corresponding to a population mean fitness of 1 is

quite large and could generate considerable selection pressure for female choice. For example, if we assume that fitness is normally distributed, then a female able to discriminate against the worst 50% of males would have a selective advantage of about 4% over one that mated indiscriminately (see below). Females accepting only the top 10% would have an 9% selective advantage. The conservative nature of the estimate of the variance of fitness suggests that these values might very plausibly be doubled, or more. (In deriving these values I have ignored the problem of how females might actually evaluate males and have assumed that there was no selective juvenile mortality.) On the other hand, field workers will find a CV^2_w equal to 0.01, or even ten times that, very small indeed. For example, if fitness is measured as the number of adult recruits produced, and the population is neither increasing nor decreasing in size, then the mean number of recruits produced will be 2. If we assume that the environmental variance is also about 2, corresponding to a Poisson distribution of recruits for individuals of mean fitness, then the expected heritability is about $0.01/(2+0.01) \cong 0.005$. Heritabilities of this magnitude cannot be distinguished from zero in an experiment of reasonable size. Values 5 times as large are at about the limit of resolution for laboratory studies on Drosophila (cf. Fig. 4 in Roff & Mousseau 1987). What is true for field workers is also true for choosy females, and adaptive mate choice, if it exists, ought to be based on criteria which are better predictors of genetic quality than realized reproductive success.

The advantage of female choice. To quantify the possible fitness advantage of eugenic female choice more precisely, it is necessary to have an algebraic expression in terms of some measurable quantities. One such formula may be derived as follows.

First consider a population of males; each male has a phenotypic attractiveness A_P and a genotypic attractiveness A_G. The selected population of males (i.e. the original population weighted by the number of offspring each one gets to father) will have a higher mean attractiveness than the baseline population. The difference in the mean attractiveness between selected and baseline populations, standardized by the standard deviation of attractiveness in the baseline population, is the intensity of selection on attractiveness, i:

$$\Delta A_P/\sigma_{AP} = i \quad .$$

To get the analogous quantity for genotypic attractiveness (i.e. the change in mean genotypic attractiveness due to sexual selection, measured in genotypic standard deviations), one has to multiply this quantity by the correlation between genotypic and phenotypic attractiveness, which is h, the square root of the heritability of attractiveness:

$$\Delta A_G/\sigma_{AG} = ih \quad .$$

Third, to get the analogous quantity for fitness (i.e. the change in mean fitness due to mate choice, measured in standard deviations of fitness), one has to multiply this quantity by the genetic correlation between attractiveness and fitness, r_G:

$$\Delta w/\sigma_w = ihr_G \quad .$$

Finally, to get the mean fitness of selected males relative to the baseline population, on must multiply this quantity by the coefficient of variation of fitness, CV_w:

$$\Delta w/w = ihr_G CV_w \qquad (2).$$

In words, the fitness of selected males relative to the baseline population is the product of the intensity of mate choice, the square root of the heritability of attractiveness, the genetic correlation between attractiveness and fitness, and the additive genetic coefficient of variation of fitness. The selective advantage of a gene coding for such choice will be half this value (Charlesworth 1987).

As seen above, Fisher's Fundamental Theorem can be used to derive an estimate of the coefficient of variation of fitness; it may also be used to estimate i, the intensity of selection on male attractiveness. The theorem states that the change in fitness relative to the mean fitness is equal to the square of the coefficient of variation of fitness. If we substitute attractiveness for fitness, we get

$$\Delta A_P/A_P = \sigma_{A_P}^2/A_P^2 \quad .$$

To get the intensity of selection we simply multiply both sides by A_P/σ_{A_P}:

$$i = \Delta A_P/\sigma_{A_P} = \sigma_{A_P}/A_P \quad .$$

The intensity of selection on attractiveness is equal to the coefficient of variation of attractiveness. This latter quantity may be estimated in the field as the coefficient of variation of male mating success in species with strong female choice. For example, in the black grouse Tetrao tetrix, a lekking bird, the coefficient of variation of male reproductive success (measured as number of copulations) has been estimated to be about 1.8 (Kruijt & de Vos 1988). This is equivalent to truncation selection in which the bottom 90% of males are rejected (Fig. 11.3 in Falconer 1981).

Thus we have estimates for two of the four quantities in equation (2). Typical values for the two other parameters, the square root of the heritability of attractiveness and the genetic correlation between attractiveness and fitness, can only be guessed at. If both are set to 0.5 (not wholly unreasonable values - cf. Mousseau & Roff 1987; Roff & Mousseau 1987; Taylor et al. 1987), then the selective advantage of a choice gene is 0.5x1.8x0.5x0.5x0.1x100% = 2.25%. This is also the expected advantage of offspring of choosy females compared to those of females not allowed to discriminate, and it is very similar to the values observed by Partridge (1980). As noted, the equilibrium genetic variance for fitness is likely to be much higher in the field than in the lab; how much higher, and whether it leads to an advantage for female choice large enough to offset its costs, cannot presently be answered. Two of the more obvious lacunae which might soon be addressed are the rate of deterioration of natural environments and the covariance of relatives with respect to mating success.

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SUMMARY & DISCUSSION

The main findings reported in this thesis, empirical and conceptual, are:

1. In mammals the excess chiasma frequency and the haploid number are uncorrelated.

   The excess chiasma frequency is positively correlated with age at maturity, even controlling for differences in either litter size or body size It is negatively correlated with litter size, and there is no significant correlation if one controls for age at maturity. The haploid number does not correlate with any life history variable tested.

   Domesticated mammals have higher excess chiasma frequencies than wild species.

2. For parasites of mammals there is a positive correlation between the number of parasite generations per host generation and the host generation time.

3. Beech seedlings suffer less leaf damage due to parasites than suckers, and this advantage decays with time.

4. There is a positive correlation between male and female chiasma frequencies.

   Though many species individually show significant sexual dimorphism for chiasma frequency, there is no evidence of consistent differences between species outside the Liliaceae.

   The magnitude of sexual dimorphism independent of sign is smaller than expected based on the hypothesis that sex differences are completely neutral, but do not differ between dioecious animals, hermaphroditic plants, and hermaphroditic animals.

   In species in which one sex has an achiasmate meiosis, there is no indication of compensation by the other sex.

   The sex bivalent in female mice has a higher chiasma frequency than the autosomes.

5. Characters evolving by independent random walks may be significantly correlated under the conventional test of association, but not under a sign test of the correlations within phylogenetically independent contrasts. (A contrast is any group of two or more species and may be represented as a path joining the member species through the phylogeny; two or more contrasts are phylogenetically independent if their paths do not meet at any point.)

   Information on the age of a contrast can be used to test hypotheses about a) punctuations at time of speciation, b) null models of evolution, and c) phylogenetic inertia.

6. Crop losses due to viruses, bacteria & fungi, weeds, nematodes, and insects are for the most part independent of one another, independent of the taxonomic affiliation of the crop, and independent of the area of cultivation, mode of propagation, and breeding system.

   Annuals suffer less damage due to viruses, weeds, and insects than perennial herbs and shrubs; trees suffer even less than annuals.

7. Increasing the genetic heterogeneity of Impatiens plants in a pot increased the phenotypic variance, but had no detectable effect on either mean yield or mean susceptibility to insect pests.

8. A quantitative genetic model of fitness is developed; it predicts negative epistasis for fitness, negative correlations for fitness between loci, and greater variance and higher mean fitness in a sexual population than an asexual population.

   The population-level advantage of sex is predicted to increase with the rate of recombination, the number of recombining loci, and the intensity of selection, and to decrease with population size.

   Fisher's Fundamental Theorem of Natural Selection and data on the rate of decay of fitness under mutation pressure in Drosophila together give a conservative estimate of 0.1 for the additive genetic coefficient of variation of fitness.

The advantage of female choice is given by the expression $Ad=ihr_GCV_W/2$, where i is the intensity of choice, h is the square root of the heritability of attractiveness, r_G is the genetic correlation between attractiveness and fitness, and CV_W is the additive genetic coefficient of variation of fitness. Fisher's Fundamental Theorem and data on variance in male mating success in the black grouse together give an estimate of 1.8 as the intensity of selection on male attractiveness.

I believe that all of these are "contributions to original knowledge".

The findings reported in this thesis do not identify a unique unifying principle which governs the population biology of sex and recombination. Nevertheless, there are some recurrent themes relating to the ideas outlined in the Preface which I should like to discuss.

The first is the idea that sex and recombination function to diversify siblings so that they compete less with each other and/or are less likely to infect one another. This hypothesis fares poorly in the studies reported in this thesis: genetically diverse crop plants were not found to suffer lower levels of pest and parasite damage (chapter 6), increasing the genetic diversity of Impatiens plants in a pot did not increase total production or reduce pest levels (7), and there is no positive correlation between rates of recombination and litter size in mammals (1). I cannot recommend this hypothesis as a basis for designing interesting and informative studies.

More promising is the idea that sex and recombination function to make progeny different from the parental generation, and so to escape from coevolving parasites. In accordance with this theory, perennial herb and shrub crops suffer more damage than annuals (6), beech seedlings show a transient reduction in leaf parasitism compared to suckers (3), and there are both more parasite generations per host generation and more chiasmata at meiosis in longer lived mammals (1 & 2). This hypothesis seems to be useful in identifying patterns in nature.

Both these theories emphasize the diversity produced by sexual reproduction, and are most easily expressed in terms of selection between families. Quite a different view of sex comes from considering the fate of a modifier gene whose only effect is to alter the level of recombination. If

such a gen, because of its effects, tends to be found on better than average chromosomes, then it may 'hitchhike' to fixation. This gene-level perspective is usually favoured by those developing formal genetic models of the evolution of recombination. It is also probably the most useful in understanding the observations that domesticated mammals seem to have higher rates of recombination than wild species (1; see also Maynard Smith 1988), that there are no consistent sex differences in recombination between species (4), and that the sex bivalent in female mice has a higher chiasma frequency than the autosomes (4). The theory presented in Chapter 8 suggests that one mechanism whereby a recombination modifier might become associated with better than average chromosomes is by increasing the response to selection for higher fitness.

Maynard Smith, J. 1988. Selection for recombination in a polygenic model - the mechanism. Genet. Res. 51:59-63.