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The evolution of alternative morphologies: an empirical investigation in the wing dimorphic cricket, *Gryllus firmus*

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A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree Doctor of Philosophy

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Abstract.-Successional changes in a habitat may result in bottlenecks where few individuals in a population survive. During such events, changes in the genetic architecture of traits are predicted to occur as is subsequent inbreeding depression. In two literature reviews, I document that, (1) dominance variance increases in traits that are subject to strong selection and, (2) inbreeding depression is substantially higher in the wild as compared to captive populations. In addition to these changes, successional pressures may also result in the evolution of morphologies that allow organisms to avoid unfavourable conditions. A common dimorphism in insects is wing dimorphism, in which the macropterous morph is long-winged (LW), has functional flight muscles and is flight-capable while the micropterous morph (SW) has reduced wings and cannot fly. Due to the energy required to maintain the flight apparatus, macropterous individuals are predicted to have less energy available for reproduction. Trade-offs to macroptery have been documented in female insects. *Gryllus firmus* is a wing-dimorphic cricket of the southeastern USA. Although there are well established trade-offs between macroptery and reproduction in female crickets, no trade-offs have been demonstrated in male crickets. The prediction is that LW males, because they have to expend energy to maintain the flight apparatus, will call less and therefore attract fewer females than SW males. To be evolutionarily important, the traits involved in the trade-off; call duration, wing morph, wing muscle condition and lipid weight should have significant heritabilities and be genetically correlated. I found that SW males attracted significantly more females than LW males (mean %=70% (SW) 30% (LW)). A significant difference in time spent calling was found between SW and LW males and as the difference in calling time between males increased, the likelihood of a female choosing the longer-calling male also increased. All the traits had significant heritabilities and most of the genetic correlations were also significant. Therefore, these results support the hypothesis that the trade-off to macroptery is an important factor in the maintenance of wing dimorphism in male *G. firmus*.

Résumé.-Dans un environnement, les changements successionnels peuvent occasionner des étranglement où seulement quelques individus survivent. Dans deux revues de littérature, j'ai démontré que, (1) la variance de dominance augmente chez les traits sujets à une forte sélection et, (2) les croîsements consanguins sont substantiellement plus élévés chez les populations à l'état sauvage que chez les populations en captivité. De plus, les pressions successionnelles peuvent occasionner l'évolution des morphologies qui permettent aux organismes d'éviter des conditions non favorables. Un dimorphisme commun chez les insectes est celui des ailes. Les individus à morphologie macroptaire (AL) possèdent des ailes longues et des muscles de vol fonctionnels leur permettant de voler. À l'opposé, ceux à morphologie microptaire (AR) possèdent des ailes et des muscles de vol réduits ne leur permettant pas de voler. Une hypothèse est que, dû à l'énergie requise pour maintenir cet équipement de vol, les macroptères auraient moins d'énergie disponible pour la reproduction. Ces compromis ont été documentés chez les insectes femelles. *Gryllus firmus* est un grillon aux ailes dimorphique squi provient du Sud-Est Américain. Même si les compromis entre les macroptères et la reproduction des grillons femelles sont établis, aucun compromis n'a été démontrés chez les grillons mâles. La prédiction est que, étant donné que le mâle à ailes longues dépense de l'énergie pour maintenir son équipement de vol, il appellera moins. Par conséquent, il attirera moins de femelles qu'un mâle à ailes réduites. Pour être évolutivement importants, les traits impliqués dans ces compromis (durée de l'appel, morphologie des ailes, condition des muscles de vol et poids de la masse lipidique du grillon) devraient démontrer une héritabilité significative et être génétiquement correlés. J'ai observé que les mâles à ailes réduites attiraient significativement plus de femelles que les mâles à ailes longues (%moyen=70% (AR), 30% (AL)). Une différence significative dans la durée de l'appel chez les mâles fût observée entre les deux morphologies. Plus la différence entre la durée d'appel de deux mâles est élevée, plus la femelle sera portée à choisir celui qui appelle le plus longtemps. Tous les traits démontrent une héritabilite significative et la plupart des corrélations sont significatives. Donc, les résultats supportent l'hypothèse affirmant que le

compromis concernant la macroptérie est un facteur important dans la maintenance du dimorphisme des ailes chez le mâle *G. firmus*.

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Remarks on Style and Authorship

Traditional and Manuscript-based Theses.

The faculty of graduate studies and research of McGill University requires that the following text be cited in full:

Candidates have the option of including, as part of the thesis, the text of a paper(s) submitted for publication, or clearly-duplicated text of a published paper(s). These texts must be bound as an integral part of the thesis.

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Additional material must be provided where appropriate (e.g. in appendices) and in sufficient detail to allow a clear and precise judgment to be made of the importance and originality of the research reported in the thesis.

In case of manuscripts co-authored by the candidate and others, the candidate is required to make an explicit statement in the thesis as to who contributed to such work and to what extent. Supervisors must attest to the accuracy of such statements at the doctoral oral defense. Since the task of the examiner is made more difficult in these cases, it is in the candidate's best interest to make perfectly clear the responsibilities of all authors of the co-authored papers. Under no circumstances can a co-author of such a thesis serve as an examiner for that thesis.

All chapters were written for publication in scientific journals. All experiments were executed by myself, but designed with the close supervision of my supervisor, Derek Roff. Professor Derek Roff contributed to the development of these projects within the normal supervisory role. In addition, Derek Roff co-authored the manuscripts presented here in this thesis.

Chapter 1 is published in Heredity (Volume 75, pages 530-540, 1995). Chapter 2 has been prepared for submission to Heredity. Chapter 3 is published in Animal Behaviour (Volume 50, pages 1475-1481, 1995). Chapter 4 is published in Animal Behaviour (volume 56, 1998). Chapter 5 is published in Evolution (Volume 52(4), 1998). Chapter 6 has been prepared for submission to Oecologia.

Original Contributions to Knowledge

This thesis examines, (1) the evolutionarily important factors that contribute to the maintenance of wing dimorphisms in male *G. firmus* and (2) the effect of selection on the genetic architecture of traits and the importance of inbreeding depression in the wild. I hypothesize that wing dimorphism is maintained in males as a consequence of the energetic trade-off between the maintenance of the flight apparatus (long wings, flight muscles and flight fuels) in macropters and the likelihood of attracting a female. To the best of my knowledge, it is the first body of work that has quantified the genetic basis to the trade-off with macroptery in a male wing dimorphic insect.

Chapter 1.

The first chapter of the thesis is a compilation of a large number of estimates of dominance variance to determine if traits subject to strong selection or closely associated with fitness have higher levels of dominance variance than traits less subject to selection pressure. Although compilations of dominance variance have been published in the past, they have either been only qualitative, or have reported only a few estimates for a small select group of species (domestic species). This is the most comprehensive compilation of dominance variance available in the literature.

Chapter 2.

Inbreeding depression has been well studied in zoo species and domestic species of economic importance. Although a well established phenomenon in captive species, the lack of estimates of inbreeding depression in wild out-bred populations has lead to the remaining skepticism in the scientific community of its importance in the wild. This review is the first compilation of estimates of inbreeding depression in wild out-bred species and the first to compare a large number of estimates of inbreeding depression between captive and wild populations.

Chapter 3.
Most of the research concerning wing dimorphisms in insects and trade-offs associated with wing dimorphisms in the scientific literature has been concerned with how trade-offs maintain wing dimorphisms in females. Using females in this research is the natural first choice since trade-offs to macroptery can be easily quantified in terms of the number of eggs laid. Because of this, trade-offs to macroptery have been well studied in females and almost completely ignored in males. This study was the first to establish that trade-offs to macroptery involving calling behaviour exist in male *G. firmus.*

Chapter 4.
Having established that a trade-off to macroptery involving calling exists in male *G firmus*, the next experiment set out to determine if variance in environmental factors that crickets may experience in the wild would affect the magnitude of the trade-off. For this study I examined how food restriction in both macropterous and micropterous crickets affected total time spent calling and subsequently the likelihood of attracting a female. This study was the first to examine how limiting resources affected the trade-off to macroptery in a male wing dimorphic insect.

Chapter 5.
Although trade-offs to macroptery in females have been well studied on a phenotypic level, only two studies (one of which includes female *G. firmus*) has established a genetic basis to the trade-off. This study was the first to establish a genetic basis to the trade-off between macroptery and reproduction in a male insect. For this study I estimated the heritability for wing morph, flight muscle weight and call duration and calculated genetic correlations between wing morph and call duration and call duration and flight muscle weight.

Chapter 6.
Having established that a macroptery-reproduction trade-off exists on both the phenotypic and genetic levels, the final study presented in this thesis determined how traits most proximate to the energetics of the trade-off (lipids, flight muscles), operate within the trade-off. Because the

macroptery-call trade-off is believed to be mediated on a physiological level by the partitioning of resources to the maintenance of the flight apparatus and calling, negative correlations between traits on this level (call duration and flight muscle weight) are predicted to be larger (more negative) than traits not as proximate to the energetics of the trade-off (call duration and wing morph). This study was the first to quantify in a male wing dimorphic insect, the fitness gain due to microptery in comparing flight muscle variation and lipid weight.

Acknowledgments

First and foremost, I want to thank my thesis supervisor Derek Roff, for being an incredibly understanding and patient man during my stay at McGill. Without his help, this thesis would never be. Secondly, I'd like to thank my committee members, Don Kramer and Martin Lechowicz for asking the hard questions. Finally I want to thank my fellow lab residents Richard Preziosi, Marc DeRose, Serge Mostowy, Martin Cayer and Vicky Goyetche for distracting me from my work. Translation of the abstract was most kindly done by Martin Cayer. This research was supported by an NSERC grant to Derek Roff.

General Introduction

Migratory insects and those that inhabit areas of constant environmental or successional change, frequently experience population bottlenecks. During succession (biotic community replacement during disturbance), insects that have the capability of migrating or moving to avoid unfavourable conditions will survive. In addition to evolving migratory strategies, insects may also evolve other life history strategies that allow them to stay in their habitat but avoid changes in the environment. In both instances, individuals may experience intense directional selection. Quantitative genetic theory predicts that with strong selection, changes in the genetic architecture of traits will occur (Falconer 1989). Strong directional selection (and to a lesser degree, stabilizing selection; Lee and Parsons 1968; Lacy 1987) is predicted to erode additive genetic variance and, consequently, decrease the heritability of a trait (Felsenstein 1965; Lande 1988; Turelli 1988; Gomez-Raya and Burnside 1990; Villanueva and Kennedy 1990; references in Arnold 1990; for a review see Rose et al. 1987). As a consequence, the response to selection will be reduced (Falconer 1989; but see Lande 1988 for an alternative explanation). Because they are assumed to be subject to intense selection (irrespective of whether or not it is because of successional changes in the environment), traits closely associated with fitness are predicted to have low heritabilities and consequently relatively high dominance variances (Wright 1929; Haldane 1932; Lerner 1954; Fisher 1958). Two reviews by Mousseau and Roff (1987) and Roff and Mousseau (1987) have shown that life history traits, which are assumed to be closely connected to fitness, have low heritabilities, while morphological traits, which are assumed to be more distantly related to fitness, have high heritabilities. Because selection usually erodes only additive genetic variance (Lynch 1994), although changes in gene frequency (as would occur during bottleneck events) may also cause changes in non-additive variance as well, one would predict that the opposite pattern should be found for dominance variance: life history traits should have relatively high levels of dominance variance while morphological traits should have low levels of dominance variance. Few studies exist that have examined changes that occur as a result of selection acting on the genetic architecture of quantitative traits. Short

compilations have been made of patterns of dominance and non-additive variance in the past but they have been either qualitative (Kearsey and Kojima 1967) or have reported quantitative results of only a few traits for a few species (see Garland 1994 for a review). One goal of this thesis will be a compilation of estimates of dominance variance from traits closely (strong selection) and distantly (weak selection) related to fitness to gain a better understanding of how the genetic architecture of traits change during successional changes in the environment and during bottleneck events.

In addition to changes in the genetic architecture of traits that occurs during bottleneck events, individuals in a population will subsequently experience high levels of inbreeding. With high levels of inbreeding, one also expects to find high levels of inbreeding depression (Wright 1977; Shields 1987). The most common estimates of inbreeding depression involve traits that are closely related to fitness such as number of eggs laid and offspring survival. Most of the literature concerning inbreeding depression has concentrated on domestic or captive-bred wild species (Ralls and Ballou 1986; for a review see Lacy et al 1993) since these animals and plants are of most practical importance. One of the most comprehensive data sets is that of zoo animals (Ralls et al. 1988); thirty-eight species showed an average increase in mortality of 33% for inbred matings (Ralls et al 1988). The total costs of inbreeding in natural populations is predicted to be considerably higher due to environmental factors (Ralls et al 1988). Although the implications of high levels of inbreeding depression to the evolution of traits and to population extinction are obvious (Lande 1988; Caro and Laurenson 1994; Caugley 1994), the degree of inbreeding depression in the wild still remains controversial (Frankham 1995). Evidence of inbreeding depression in wild species exists (see Frankham 1995 for examples), but the lack of a comprehensive review has lead to the remaining skepticism of its existence in natural populations (Caro and Laurenson 1994). In addition to compiling estimates of dominance variance, I have also gathered estimates of the magnitude of inbreeding depression from wild populations to determine if it is of evolutionary importance and if it is substantially higher than has been estimated in captive populations.

Successional changes in a habitat may result in the evolution of morphologies that allow organisms to avoid or minimize the detrimental effects of environmental change. Most insects (99%) belong to the winged super order Pterygota. Flight capability is an ideal means by which to avoid unfavourable changes in the habitat. Despite this, certain genera are composed of species that are monomorphically wingless or dimorphic for wing length. Wing dimorphism is a condition where one morph (macropterous) possess a functional flight apparatus and the other (micropterous) is flightless. Wing dimorphism has evolved independently in most of the major orders of insects (Harrison 1980; Roff 1986a, 1994). Wing dimorphism can take on many types of distribution between and within the sexes (for a review see Thayer 1992; Roff 1994). Of particular importance to the study of trade-offs and the evolution of winglessness is the study of intrasexual dimorphisms. Intrasexual dimorphisms allow us to examine how such alternate morphologies evolve by determining what associated behavioural and life history differences exist between morphs and how these differences contribute to differences in fitness. Intersexual dimorphisms are confounded by differences between the sexes, and therefore results obtained from such investigations are not always clear.

The obvious advantage to being long-winged and flight capable is the ability to move over large areas and in three dimensional space or to avoid environmental changes in a habitat (Roff 1990). Although in some cases of dimorphic variation, one morph may have a reduced fitness and be adopting the "best of a bad lot" strategy (Eberhardt 1982), the wide occurrence of dimorphisms suggests that, in general, the dimorphism is maintained in the population as a consequence of trade-offs (Gross 1984; Roff 1984; Hazel et al. 1990; Roff and Fairbairn 1994). The existence of wing dimorphism suggests a trade-off between resources devoted to dispersal (wings, flight muscles and flight fuels) and those devoted to reproduction (Roff 1984, 1986a; Crespi 1988a; Roff and Fairbairn 1991; Denno et al. 1991; Zera et al. 1994). Although producing and maintaining the long wings that macropters possess is probably not energetically significant, the production and maintenance of the massive flight muscles and flight fuels is energetically expensive (Roff 1989; Mole and Zera 1993; Tanaka 1993; Stryer 1988). In addition to large flight

muscles, functional muscles (indicated by a red colour for presence of mitochondria and cytochrome; Ready and Josephson 1982; Shiga et al. 1991; Gomi et al. 1995) are believed to be much more costly to maintain than partially or non-functional muscles (Mole and Zera 1993; for a review see Zera et al. 1997).

Among wing dimorphic insects, trade-offs between macroptery and reproductive potential have been well studied in females. Although no consistent pattern with respect to adult longevity or development time has been documented between morphs, differences have been found in age at first reproduction and overall fecundity. Micropterous females reproduce earlier and have a higher fecundity than macropterous females (Roff 1986b; Denno et al. 1989; Roff and Fairbairn 1991; Mole and Zera 1993). These differences have been shown to be very large in the case of female sand crickets, *Gryllus firmus*: micropterous females reproduce significantly earlier and produce 60% more eggs in a 6-week period than do macropterous females (Roff 1984). Physiological studies have shown that fecundity differences in *G. firmus* females can be ascribed to the allocation of resources to reproduction in the SW morph and those to flight apparatus maintenance in the LW morph (Mole and Zera 1994). In addition, genetic correlations between wing morph and fecundity have been demonstrated in female *G. firmus* (Roff 1990b; 1994) and the cricket *Allonemobius socius* (Roff and Bradford 1996; Roff et al. 1997), indicating that the trade-off will modulate the evolution of these two traits.

Although the existence of negative phenotypic correlations between wing morph and reproduction have been well documented in female insects, few examples of such general trade-offs have been found in male insects. Male *Hoplothrips karnyi* are characterized by a double dimorphism, a foreleg dimorphism and wing dimorphism. The foreleg dimorphism is correlated with the wing dimorphism, wingless males having enlarged forelegs and winged males small forelegs (Crespi 1988a). Males with the larger forelegs win fights more often and become dominant at oviposition sites (Crespi. 1988a,b). Utida (1972) indicated that in the coleopteran *Callosobruchus maculatus*, the most frequent copulations occurred between wingless males and females. Unfortunately Utida did not include any data to support this observation.

Brachypterous (short-winged) males of the planthopper *Nilaparvata lugens* are reported to develop earlier and outcompete macropterous males (Novotny 1994). Fujisaki (1992), has shown that a fitness advantage exists to wing reduction in male *Cavelerius saccharivous* in terms of earlier sexual maturity, leading to a greater proportion of matings in favour of the brachypterous (wing absent) morph. Although the above mentioned studies have demonstrated phenotypic trade-offs between morphological and life history traits in male insects, to date no study has examined if the traits involved in the trade-off have a genetic basis and if the trade-offs themselves are genetically based.

In *G. firmus* no general trade-offs in males have been found. Roff and Fairbairn (1994) found no differences in testes size or proportion of offspring sired between morphs of *G. firmus*. But a difference was found in development time, macropterous males developing earlier than micropterous males. This is contrary to the proposed trade-off hypothesis and therefore cannot account for the preservation of the two morphs in males (Roff and Fairbairn 1994).

Although no fitness differences have been found to account for the preservation of two morphs in male *G. firmus*, selection acting on females alone may be all that is required to determine proportion macroptery in males. A high intersexual genetic correlation may prevent the independent evolution of the trait in question in either sex (Via and Lande 1985). Although an independent optimum may be reached, the time required to do so will usually be very long since selection acting on one sex will affect the trait in the other. Roff and Fairbairn (1994) found the intersexual genetic correlation for wing form in *G. firmus* to be quite high (0.86). This high correlation is in accord with other estimates of the genetic correlation of morphological traits between the sexes (Price and Burley 1993; see table 5, Roff and Fairbairn 1994). But unless a correlation is exactly $\pm$ 1, selection will still favour the evolution of wing form to a separate optima in each sex (Via and Lande 1985).

For male *G. firmus,* despite the fact that no general costs to macroptery were found, an overview of the distribution of the frequency of macroptery between the sexes in wing dimorphic insects suggests that a trade-off exists. In most insect orders comprised of species that are wing dimorphic, the distribution of macroptery between the sexes is either

equal for males and females (Coleoptera), or only slightly biased towards males (Homoptera and Hemiptera). In the Orthoptera (grasshoppers and crickets) on the other hand, 86% of the species show a female bias in proportion macroptery (see Table 5 in Roff and Fairbairn 1994). The Orthoptera differ from other orders in that mate attraction involves calling by the males. The female biased distribution suggests that macroptery in males may be more costly than in females and that a trade-off between macroptery and calling behaviour exists (Roff and Fairbairn 1994).

In *G. firmus*, as with many other Orthopterans, males are relatively sedentary as adults (Alexander 1968; Dadour 1990; Cade and Cade 1992) and call to attract females to mate. A number of components of calling are known to be important cues for female attraction, of which one of the most important seems to be total time spent calling (Table 1). Studies on Orthopterans have shown that the time spent calling is typically directly proportional to the likelihood of attracting a female (see Table 1 for references). Calling is an energetically demanding behaviour requiring on average a tenfold increase in metabolic rate (data for seven species from Table 1 of Bailey et al. 1993; and one species from Forrest 1991b). Producing and maintaining the flight apparatus that macropterous crickets possess is energetically expensive (Roff 1989; Mole and Zera 1993; Tanaka 1993). It is not known if resources required for wing production, flight fuel production and flight muscle formation and their maintenance, constrain those needed for calling. If such a constraint exists, then a trade-off between macroptery and calling may promote the maintenance of the two morphs in male *G. firmus*.

This thesis will test the hypothesis that a trade-off exists between flight capability and the probability of attracting a female. Such a trade-off is predicted to be reflected in differences between micropterous and macropterous male *Gryllus firmus* in the likelihood of attracting a female: micropterous males are predicted to attract more females than macropterous males. I hypothesize that such a difference is a consequence of time spent calling. If the energy needed for the formation and maintenance of the flight apparatus competes with that needed for calling in macropterous males, micropterous males are predicted to call longer during a 24 hour period than macropterous males. In order for the trade-off to be relevant in the wild, it must exist

under a variety of environmental conditions (Stearns 1989). Because the trade-off in *G. firmus* is believed to be an energetically mediated one where resources are partitioned between flight muscle maintenance and calling, then with variation in available resources, I predict that the magnitude of the trade-off will change. In addition, in order for such a trade-off to have any evolutionary consequence to the evolution of the traits involved in the trade-off, wing morph, call duration, flight muscle weight and lipid weight must have a genetic basis and the relevant correlations between the traits must also have a genetic basis. While in *G. integer,* call bout length has a significant and high heritability (0.75; Hedrick 1988, 1994) and mean nightly time spent calling also has a significant heritability (0.50; Cade 1981), it is not known if call duration in *G. firmus* has a significant heritability. Finally, although examining the trade-off between wing morph and calling on the phenotypic and genetic levels is crucial to the understanding of how wing dimorphism is maintained in male *G. firmus*, the study would not be complete without examining how the traits most proximate to the energetics of the trade-offs (flight muscle condition, flight muscle weight and lipid weight) operate within the trade-off. To the best of my knowledge, this is the most extensive study of wing dimorphisms in a male insect and the first to have examined the genetic basis of trade-offs to macroptery in a male insect.

Table 1. Examples of components of call parameters in the Orthoptera found to be important in attracting females.

call component	species	reference
call duration	*Gryllus integer*	French and Cade 1987
	Neonemobius sp.	Cade and Cade 1992
	Gryllus campestris	Forrest 1991
		Hissmann 1990
		Walker 1983
	Cyphoderris stepitans	Simmons 1988
	Gryllus bimaculatus	Snedden & Sakaluk 1992
	Omocestus viridulus	
	Chorthippus brunneus	Bohm et al. 1991
	Gryllus firmus	Eiriksson 1994
		Butlin et al. 1985
		Crnokrak & Roff 1995, 1998
song intensity	*Scapteriscus sp.*	Forrest 1983
	Gryllus integer	Cade and Cade 1992
	Gryllus pennsylvanicus	Ciceran and Murray 1994
call bout length	*Gryllus integer*	Hedrick 1986, 1994
		Hedrick and Dill 1993
vibratory signals	*Gryllus bimaculatus*	Weidmann & Keuper 1987
syllables per phrase	*Scudderia curvicauda*	Tuckerman et al. 1993

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Chapter 1. Dominance variance: associations with selection and fitness

In the first chapter of this thesis, I have compiled estimates of the relative importance of dominance variance (V_d) compared to additive (V_a) and phenotypic variance (V_p) for 16 animal species and 22 plant species. In this chapter, I answer the questions 1) do traits closely related to fitness (life history) have relatively high levels of V_d compared to traits distantly related to fitness (morphology) and 2) do traits subject to strong directional selection (domestic species) have higher levels of V_d compared to traits believed to be subject to weaker selection (wild out-bred species). These selection pressures are believed to be important evolutionary factors operative during successional changes in a habitat. This chapter has been published in Heredity (volume 75, pages 530-540, 1995).

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Dominance variance: associations with selection and fitness

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Strong directional, and to some degree stabilizing, selection usually erodes only additive genetic variance while not affecting dominance variance. Consequently, traits closely associated with fitness should exhibit high levels of dominance variance. In this study we compile a large number of estimates of dominance variance to determine if traits that are subject to strong selection and/or are closely associated with fitness have higher levels of dominance variance than traits less subject to selection pressure. Estimates were taken from the literature for both wild and domestic species and each group was treated separately. Traits closely associated with fitness (life history) had significantly higher dominance components than did traits more distantly related to fitness (morphology) for wild species. No significant differences were found between life history and morphological traits for domestic species. Traits that were known to have been subject to intense directional selection (morphological traits for domestic species) had significantly higher dominance estimates than did traits that were assumed not to have been subject to strong selection (morphological traits for wild outbred species). The results are discussed with respect to the maintenance of heritable variation and the bias introduced in the calculation of the full-sib heritability estimate by high levels of dominance variance.

Keywords: dominance variance, fitness, genetic variation, selection.

Introduction

The genetic architecture of a quantitative trait is composed of additive (V_a), dominance (V_d), epistatic (V_i) variance and interactions of all three components (Bulmer, 1985). Of most importance to the transmission of a trait from one generation to the next and for predicting the short-term response to selection is the narrow sense heritability, h^2, the ratio of additive variance to phenotypic variance. Dominance variance is generally not considered important as it does not predict the response to selection (Fisher, 1930; Lynch, 1994). However, dominance variance can affect the heritability of traits during bottleneck events where nonadditive genetic variance (both dominance and epistatic) can be 'converted' into or affect additive genetic variance and therefore become available for selection to act upon (for a review see Carson, 1990). General patterns of dominance variance have been predicted by a number of theoreticians (Lynch, 1994); although mechanisms of the evolution of genetic dominance are different among the theories, all agree that traits closely associated with fitness should exhibit high levels of dominance variance. Despite this, few studies have examined the prevalence of dominance variance in traits of evolutionary significance and how selection influences the level of dominance for traits closely and more distantly related to fitness.

Strong directional selection (and to a lesser degree, stabilizing selection; Lee & Parsons, 1968; Lacy, 1987) is predicted to erode additive genetic variance and, subsequently, decrease the heritability of a trait (Felsenstein, 1965; Lande, 1988; Turelli, 1988; Gomez-Raya & Burnside, 1990; Villanueva & Kennedy, 1990; references in Arnold, 1990 and Garland & Bennett, 1990; for a review see Rose *et al.*, 1987). As a consequence, the response to selection will be reduced (but the reduction in response may result from other factors: see Falconer (1989) and Lande (1988)). Traits for a population at equilibrium are predicted to have low additive genetic variance as it is assumed that selection has moulded them to an optimum (Hegmann & Dingle, 1982; Lynch & Sulzbach, 1984; but see Charlesworth (1987) for an alternative explanation). Because they are assumed to be subject to intense selection, traits

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most closely associated with fitness are predicted to have low heritabilities and subsequently relatively high dominance components (Wright, 1929; Haldane, 1932; Lerner, 1954; Fisher, 1958). A number of studies (Mousseau & Roff, 1987; Roff & Mousseau, 1987; Garland *et al.*, 1990; Willis *et al.*, 1991; Partridge, 1994) have shown that life history traits, which are assumed to be closely connected to fitness, have low heritabilities while morphological traits, which are assumed to be more distantly related to fitness, have high heritabilities (behavioural and physiological traits are usually intermediate to the above two types of traits). Because selection usually erodes only additive genetic variance (Lynch, 1994), although changes in gene frequency may also cause changes in nonadditive variance as well, one would predict that the opposite pattern should be found in terms of dominance variance: life history traits should have relatively high levels of dominance variance while morphological traits should have low levels of dominance variance. In addition to eroding additive variance, selection is also expected to act directly on genetic dominance, resulting in a further relative increase of dominance variance to total genetic variance (Mather, 1979; Lynch, 1994). Fisher's (1958) theory predicts that dominance will evolve in both magnitude and direction of increasing fitness. Although Fisher's theory of the evolution of dominance has been strongly criticized (for a review see Charlesworth, 1979), empirical investigations have confirmed the last point (Breese & Mather, 1960; Kearsey & Kojima, 1967; Lynch, 1994 and references therein). On a per species basis, few studies exist that have examined changes that occur as a result of selection acting on the genetic architecture of quantitative traits. For the most part, strong selection does decrease additive variance and, in most cases, heritability, although in some cases the decreases are relatively small (Kaufman *et al.*, 1977; Enfield, 1980). Discrete traits such as dimorphisms that are controlled by some underlying normally distributed variable, when subject to strong directional selection may not experience an appreciable decrease in genetic variance because the intensity of selection declines very quickly over time (Roff, 1994).

Short compilations have been made of patterns of dominance and nonadditive variance in the past but these have been either qualitative (Kearsey & Kojima, 1967) or have reported quantitative results of only a few traits for a few species (see Garland, 1994 for a review). This study is the first to attempt a compilation of a large number of estimates concerning the relative distribution of dominance variance across traits that are associated with fitness and/or subject to selection to different degrees.

The present study addresses two main questions. (i) How does the relative contribution of dominance variance change from one trait-type to another? Do traits that are closely associated with fitness have relatively high levels of V_d as predicted from theory? (ii) Do patterns of V_d change from wild outbred species to domestic species for particular trait categories? Although we have no studies that have examined what changes occur in the genetic architecture of a trait in the presence of selection for a wide variety of traits and species, the above comparison will allow us at least to speculate how strong directional selection can change the relative contribution of V_a and V_d to the genetic variance of a trait.

Materials and methods

Estimates of dominance variance

Three hundred and thirty-eight estimates of dominance variance were obtained from 55 sources; 17 wild outbred species and 21 domestic species. Species were classified as domestic if they had been subject to long-term deliberate artificial selection and usually included species of economic importance such as *Zea mays*. The relative contribution of dominance variance to the genetic variance of a trait was calculated in two ways: (i) dominance variance as a function of the sum of dominance and additive variance:

$$D_z = \frac{V_d}{V_d + V_a},$$

and (ii) dominance variance as a function of phenotypic variance:

$$D_\beta = \frac{V_d}{V_p}.$$

The variable D_z was used to assess the relative contributions of dominance and additive variances irrespective of the phenotypic variance for a trait. Although selection may affect V_p (Hayashi & Ukai, 1994), most interest lies in how it affects the genetic architecture of a trait. Nevertheless, a high level of V_d may be inconsequential to a trait if it is small compared with V_p; for this reason, we also used the variable D_β. Negative variance estimates reported in the literature were standardized to zero (Kendall &

Stuart, 1979). We obtained 232 estimates of D_z and 106 estimates of D_β. For those studies that reported more than one estimate of dominance variance for each trait (repeated trials or measures under different conditions) we used the mean. A list of species, estimates and references is available from the corresponding author upon request. This is the most extensive compilation of this sort to date: it is representative but not exhaustive.

Estimates were categorized as belonging to one of four groupings: (i) behavioural traits; (ii) life history traits; (iii) morphological traits; and (iv) physiological traits. Although somewhat ambiguous in some cases, we restricted life history traits to those traits that are thought to be most closely associated with fitness. For example, life history traits included development time, flowering time and lifetime fecundity. Morphological traits referred to metric size or quality measurements such as body weight, bill colour and seed weight. Examples of behavioural traits are proboscis extension behaviour, locomotion rate and escape–avoidance conditioning. Physiological traits included both metabolic processes such as freezing resistance, and the biochemical composition of substances such as seed protein content.

The analysis addressed two main questions. (1) Are there differences between trait categories for both measures of dominance? In conjunction, are the estimates biased because of over-representation of species and/or characters? If this is the case, then if significant differences exist between life history and morphological traits, are they maintained after correction for this bias? (2) Are there statistical differences between wild and domestic species in terms of the relative importance of dominance variance for complementary trait categories?

Statistical analysis and results

Predictions

Although a number of different theories exist to explain the evolution of genetic dominance, most agree that traits associated with fitness will tend to exhibit relatively high levels of dominance variance (Fisher, 1928; Wright, 1929; Haldane, 1932; Dobzhansky, 1952; Lerner, 1954; Kacser & Burns, 1981). Consequently, one would expect that life history traits, as they are closely related to fitness, should exhibit high levels of dominance variance in wild populations. Low levels of dominance variance are predicted for traits distantly related to fitness such as morphological traits. From previous theoretical (Lee & Parsons, 1968) and empirical (Falconer, 1981, table 10.1, p. 150; Mousseau & Roff, 1987) investigations concerning the relationship between fitness and heritability estimates, one would expect that behavioural and physiological traits should fall between life history and morphological traits (Roff & Mousseau, 1987). For behavioural traits at least, this is a valid assumption as Lee & Parsons (1968) proposed that such traits will be subject to strong stabilizing selection which would thus decrease heritability estimates for this class of traits. Making predictions about domestic species is more difficult as most were subject to strong directional selection at some point. Because of this, the only *a priori* prediction concerning dominance variance is that traits that are known to have been subject at one time to strong directional selection should exhibit high levels of dominance variance. The domestic data set is composed mostly of morphological traits indicating the importance of these traits in domestic breeding programmes. We therefore predict that morphological traits for domestic species should exhibit much higher levels of genetic dominance than such traits in wild outbred species.

I. Dominance variance and fitness

Because the reported estimates of D_z and D_β are proportions, they probably violate the assumptions of parametric analysis (Sokal & Rohlf, 1981). We therefore used nonparametric analyses to test for differences between trait categories. For the following analyses wild and domestic species were treated separately. Statistical tests for differences of estimates of dominance variance across trait categories were conducted by using the Kolmogorov–Smirnov & Kruskal–Wallis tests (Wilkinson, 1990). In addition parametric ANOVA and Tukey HSD tests were used but the results for these were only reported when they differed from the above tests.

Wild outbred species Table 1(a) gives the results of the nonparametric tests conducted for wild outbred species for both measures of dominance (D_z and D_β). For the variable D_z, significant differences exist between different trait categories: Kruskal–Wallis test (D_z: $H = 15.394$, $P < 0.002$, $n = 75$; D_β: $H = 6.003$, $P < 0.111$, $n = 60$) and the Kolmogorov–Smirnov test (Table 1a). Life history traits had significantly larger dominance components than did behavioural, physiological and morphological traits (see Table 1a, and Fig. 1a for the cumulative distributions). Behavioural and physiological traits, although not statistically different from morphological traits, had means that fell between life history

Table 1 Summary of descriptive statistics and Kolmogorov–Smirnov tests for the variables D_z (below diagonal) and D_β (above diagonal) for wild outbred and domestic species

	Life history	Behaviour	Physiology	Morphology
(a) Wild outbred species				
D_z				
n	22	12	18	26
$\bar{x} \pm SE$	0.544 ± 0.072	0.237 ± 0.061	0.266 ± 0.068	0.166 ± 0.036
D_β				
n	20	2	16	24
$\bar{x} \pm SE$	0.313 ± 0.069	0.040 ± 0.020	0.209 ± 0.072	0.133 ± 0.035
Kolmogorov–Smirnov P-value (Tukey HSD)				
Life history	—	0.600	0.023 (0.616)	0.116
Behaviour	0.030 (0.011)	—	1.000	1.000
Physiology	0.040 (0.008)	0.326	—	0.464
Morphology	0.0001	0.424	0.189	—
(b) Domestic species				
D_z				
n	24		29	101
$\bar{x} \pm SE$	0.392 ± 0.058		0.479 ± 0.059	0.422 ± 0.028
D_β				
n	6		10	28
$\bar{x} \pm SE$	0.040 ± 0.026		0.307 ± 0.081	0.190 ± 0.033
Kolmogorov–Smirnov P-value				
Life history	—		0.038 (0.025)	0.011 (0.196)
Physiology	0.424		—	0.181
Morphology	0.901		0.531	—

Where ANOVA tests revealed results different from the K–S test the values are given in parentheses (Tukey HSD comparisons).

and morphology traits. No significant differences were found between trait categories for the variable D_β except for the paired comparison Kolmogorov–Smirnov test between life history and physiology. Although having lower mean values, D_β estimates were comparable to D_z estimates for all categories except behaviour (but this may result from the low sample size of two). The parametric and nonparametric tests agree for the most part except for the comparison between life history and physiological traits for D_β (see Table 1a), in which the nonparametric tests show significance but the parametric do not, suggesting differences in the shape of the curve.

Domestic species Table 1(b) presents the results of the nonparametric tests for domestic species. As no estimates were available for behavioural traits, no comparisons between this trait and the other three were possible. No significant differences were found between trait categories for the variable D_z (Kruskal–Wallis test: $H = 1.167$, $P < 0.558$, $n = 155$). A significant difference was found for D_β (Kruskal–Wallis test: $H = 8.844$, $P < 0.012$, $n = 45$); life history traits had significantly lower dominance components than did physiological traits (see Table 1b).

II. Analysis of study means

The above analysis may be biased because of the disparity in the number of estimates reported for each study (i.e. differences may result from one study with an inordinately large number of estimates). To circumvent this problem, we used single estimates for each trait category from each study. Where more than one estimate was reported for each trait-type (eg. morphology) for a given study, an average was used. This was done for estimates calculated using the variable D_z only, as not enough estimates were available for D_β. Kolmogorov–Smirnov and Kruskall–Wallis tests were used to deter-

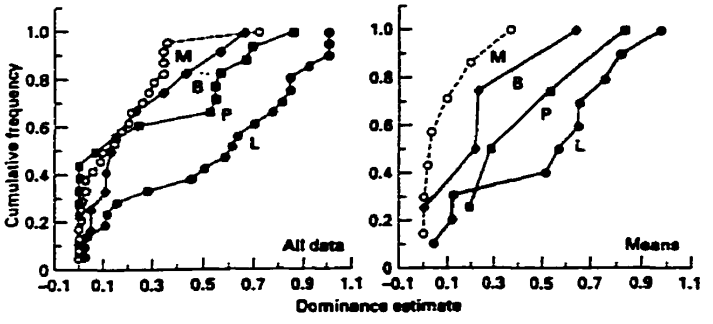

Fig. 1(a) Cumulative frequency distributions for all four trait categories (M: morphology; B: behaviour; P: physiology; and L: life history) for wild outbred species. (b) Cumulative frequency distributions for mean values calculated for all four trait categories from each study (M: morphology; B: behaviour; P: physiology; and L: life history) for wild outbred species.

Table 2 Summary of descriptive statistics and Kolmogorov–Smirnov test for study means using the variable D_s for wild outbred (below diagonal) and domestic (above diagonal) species

	Life history	Behaviour	Physiology	Morphology
Wild outbred				
n	10	4	4	7
$\bar{x} \pm$SE	0.585 ± 0.095	0.275 ± 0.136	0.528 ± 0.123	0.097 ± 0.052
Domestic				
n	14	0	21	22
$\bar{x} \pm$SE	0.396 ± 0.064	—	0.456 ± 0.069	0.395 ± 0.040
Kolmogorov–Smirnov P-value				
Life history	—	—	0.885	0.815
Behaviour	0.587	—	—	—
Physiology	0.745	0.405	—	0.719
Morphology	0.011	0.309	0.163	—

mine if significant patterns still existed as compared to the above results. Table 2 and Fig. 1(b) show that the significant differences found between life history and morphology traits for the entire data set are still maintained when study means are used for wild outbred species: Kruskal–Wallis test; $H = 9.879$, $P < 0.020$, $n = 27$ (see Table 2 for test statistics). As for the entire data set, the same results hold.

III. Analysis of paired comparisons of life history and morphological traits

To determine if the above patterns of dominance components were biased as a result of the over-representation of species or the over-representation of multiple traits measured for a given species, we chose individual studies that reported both life history and morphology estimates for a single species. As the only *a priori* prediction that can be made among the four trait categories with respect to associations with fitness is for life history and morphological traits, only these were used. We collected estimates from five wild outbred and eight domestic species. Where more than one estimate was reported for life history and morphology traits, a mean value was used in the analysis. A paired-samples t-test was used to assess if the differences between life history and morphology traits were significant.

Low sample sizes prevented the calculation of mean dominance estimates for D_β. Paired-sample t-tests revealed no significant differences between mean values of life history and morphological traits for either wild or domestic species (wild: $t_4 = -1.328$, $P < 0.255$; domestic: $t_7 = -1.102$, $P < 0.307$). Although nonsignificant, the mean difference (morphology–life history) between the mean life history and morphology values for wild species

was quite high, $D = -0.222$, and in the predicted direction (life history higher than morphological traits). Because of the low sample sizes ($n = 5$ for wild outbred species and $n = 8$ for domestic species), the results obtained for both data sets should be viewed with caution.

IV. Comparison of wild out bred and domestic species

In this section, we address the question: do D_x estimates vary between wild and domestic species? Because the literature concerning domestic species is fairly well detailed, selection pressures are known. This gives us a unique opportunity to make inferences about how strong directional selection changes the genetic architecture of traits. Of particular importance in this regard is the comparison of morphological traits between wild and domestic species as it is this trait-type that is of most importance in domestic breeding programmes (sample size for morphological traits of domestic species = 101, for all other trait categories combined = 53).

An independent samples t-test was used to determine if there exist differences between domestic and wild species in terms of dominance variance for complementary traits. Nonparametric Kolmogorov–Smirnov tests were used for paired comparisons between life history, physiological and morphological traits between wild and domestic species.

Parametric independent samples t-tests and nonparametric Kolmogorov–Smirnov paired comparisons were conducted on the three trait categories, life history, physiology and morphology for the variable D_x only, as not enough estimates were available for D_R. Because *a priori*, we predict that domestic species estimates of dominance will be higher than wild outbred species estimates using morphological traits, the test was one-tailed. Table 3 lists the test results of the paired comparisons between wild and domestic species for both parametric and nonparametric tests: morphological traits were significantly different with estimates for domestic species being higher than wild species ($t_{123} = -4.069$, $P < 0.00005$, see Fig. 2 top panel), life history traits were not significantly different between wild and domestic species ($t_{45} = 1.74$, $P < 0.089$, see Fig. 2 middle panel) and, finally, physiological traits were significantly different with estimates for domestic species being higher than wild species ($t_{46} = -2.532$, $P < 0.015$, see Fig. 2 bottom panel). The Kolmogorov–Smirnov tests tended to be more conservative than the independent samples t-tests where the only significant difference between wild and domestic species was for morphological traits (see Table 3).

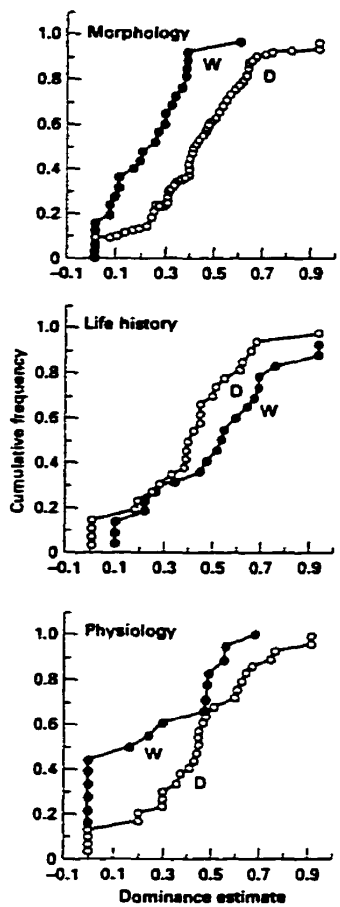

Fig. 2 Comparison of cumulative frequency distributions of wild (W) and domestic (D) species for morphological, life history and physiological trait estimates. Note: variable used here was D_x.

Discussion

Our analysis supports the hypothesis proposed by a number of theoreticians concerning selection and traits related to fitness outlined in the introduction of this paper (references therein). Traits closely associated with fitness (life history traits) were found

Table 3 Summary of mean values, independent samples *t*-tests, and Kolmogorov–Smirnov tests for life history, physiology and morphology traits between wild and domestic species

	Wild outbred $\bar{x} \pm SE$	Domestic $\bar{x} \pm SE$	*P*-value (*t*-test)
Trait category			
Morphology	0.166±0.035	0.422±0.028	<0.00005
Life history	0.544±0.073	0.392±0.058	0.089
Physiology	0.266±0.068	0.479±0.059	0.015
Kolmogorov–Smirnov test	D_{max}		*P*-value
Morphology	0.572		<0.0001
Life history	0.316		0.177
Physiology	0.336		0.131

Note: the variable used here was D_n, and the *t*-test for the morphological comparison was one-tailed.

to have significantly larger dominance variance components than traits more distantly related to fitness (morphological traits) in wild species. These differences are maintained even when mean values are used. Because of low sample size, the nonsignificant results obtained from the paired comparisons analysis of life history and morphology traits for wild species must be viewed with caution. The relative contribution to genetic variance as a result of dominance was on average higher than that of additive variance for life history traits in wild outbred species. The nonsignificant differences (both individual values and mean estimates) found between life history and morphology traits in domestic species also lends support to theory as most captive breeding programmes concentrate on morphological traits, as is evidenced by the large representation of this category of trait in the literature. Further support of the theory comes from the comparison of wild and domestic species for morphological traits alone; the significantly larger mean value of D_n in domestic species indicates that traits subject to intense selection will have a greater proportion of genetic dominance contributing to genetic variance than those that are not.

Life history traits have levels of dominance variance that on average may be as high or even higher than additive variance. Although dominance variance is inconsequential for certain methods of estimating heritability (half-sib, parent–offspring, *PO*), it may bias the h^2 estimate for full-sib (*FS*) calculations. *FS* estimates are most commonly available for natural populations of vertebrates (Brodie & Garland, 1994) as paternity cannot be assessed for gravid females taken from the wild. These estimates include some dominance, epistatic and common-family environment (maternal) effects. By standardizing the environment during rearing or using a split-cage design, one can estimate, and thus eliminate, the common family environment variance (Falconer, 1989; Arnold, 1994), but the same cannot be said of dominance variance. For a *FS* design the resemblance between siblings is measured as the intraclass correlation, *t*:

$$t = \frac{\sigma_b^2}{\sigma_b^2 + \sigma_w^2},$$

where σ_b^2 = variance between groups and σ_w^2 = variance within groups. The intraclass correlation describes the average level of relatedness between members of a group. To calculate a *FS* heritability the intraclass correlation must be multiplied by a factor of two. The *FS* estimate then includes (Becker, 1992):

$$\frac{V_a + \frac{1}{2}V_d + \frac{1}{2}V_i + 2V_{cc}}{V_p}.$$

Assuming V_i (epistatic variance) and V_{cc} (common family environment variance) contribute little to the h^2 estimate, dominance variance may significantly bias the heritability estimate in a *FS* analysis. Although heritability estimates of morphological traits will probably not be strongly biased because of dominance variance, life history traits almost certainly will. With dominance variance being on average greater than additive variance (Table 1a), life history traits that have a moderate to high heritability (i.e. h^2 over 0.60) may have a *FS* heritability that

is inflated by over 30 per cent (e.g. when $h^2 = 0.60$ the estimate will give $h^2 = 0.90$). As is evident, the *FS* heritability estimate does not represent pure additive genetic variance as a function of total phenotypic variance (Falconer 1989; Arnold, 1994; Garland, 1994). Despite this, Mousseau & Roff (1987) found no significant differences between *FS* and *PO* regression estimates in a number of taxa. Nonetheless, their conclusions that the contribution of dominance and common environment effects are low is premature as their data set was limited primarily to morphological traits (27 of 33). Assuming low or no common environment effects, one would not expect significant differences between *FS* and *PO* estimates for morphological traits as dominance variance contributes little to these types of traits (h^2 inflation because $V_d < 10$ per cent). Although it is not known to what degree total nonadditive genetic effects differ in magnitude among traits (Shwartz & Herzog, 1994; Dohm & Garland, 1993), the contribution of dominance variance will vary depending on the type of trait examined (this study; Dohm & Garland, 1993). Therefore, before deciding on a particular breeding design for estimating heritabilities, it is critical to take into consideration the types of traits being measured to obtain accurate estimates.

Most researchers discount the contribution dominance plays in the genetic architecture of traits as it is only additive genetic variance that predicts the short-term response to selection. Although this is true, dominance variance can play an important role in the maintenance of additive genetic variance. Heterozygote advantage has long been recognized as a potential means by which heritable variance is maintained (Fisher, 1958; Falconer, 1981; Lacy, 1987; for a review see Futuyma, 1986; for counter arguments see Lande, 1988). With dominance and over-dominance (greater phenotypic expression of the heterozygote over both homozygotes) at a number of loci controlling a quantitative trait such as growth rate, intense directional and to some degree stabilizing selection, will not erode as much additive variance as it would if the trait were controlled by a number of alleles whose actions were purely additive (Willis & Orr, 1993). Although in this study we report the magnitude of dominance variance and not the direction, it is not unreasonable to assume that the direction of such dominance effects would be in the direction of increasing fitness (Fisher, 1958; Lynch, 1994). The high levels of dominance variance found for life history traits could be a means by which such traits, although closely associated with fitness, may retain significant levels of additive genetic variance in the presence of selection.

The presence of high levels of dominance variance may not only be a means by which heritable variance is maintained but under certain conditions may increase the amount of additive genetic variance that is available for selection. 'Classical' theory assumes that bottleneck events are expected to decrease the additive genetic variance to a level expected from inbreeding (Crow & Kimura, 1970; Nei *et al.*, 1975; Lande, 1980; Falconer, 1981; Barton & Charlesworth, 1984; Carson, 1990). Recent theoretical (Carson, 1990; Willis & Orr, 1993) and empirical investigations (Carson & Wissotzkey, 1989; López-Fanjul & Villaverde, 1989; Bryant & Meffert, 1991) have revealed that severe bottleneck events such as those where only one pregnant female survives ($N_e = 2$), may increase the level of heritable variation available for selection. Although the proposed mechanisms by which this could occur have not been empirically verified, it is assumed that the realized increase in additive genetic variance comes about by the erosion of epistatic genetic variance (through allelic fixation at regulatory loci) and the subsequent expression, and availability to selection, of previously masked additive variance (Goodnight, 1987; Cockerham & Tachida, 1988; Tachida & Cockerham, 1989; for a review of mechanisms see Carson, 1990). Theoretical analyses have shown that unless alleles are completely additive in their heterozygous effects, population bottlenecks will increase the additive genetic variance (and subsequently, heritability) of a trait (Robertson, 1952; Willis & Orr, 1993). It is well known that dominance variance at many loci affects a number of fitness components (Simmons & Crow, 1977; Crow & Simmons, 1983; Charlesworth & Charlesworth, 1987). In the past the erosion of epistatic variance was considered the prime cause of increase in additive genetic variance in a number of empirical studies (Bryant *et al.*, 1986; López-Fanjul & Villaverde, 1989). However, recent considerations have now placed a greater importance on the effects of genetic dominance acting through changes in gene frequency as the cause (Willis & Orr, 1993). Although such effects may be transient, the high estimates of dominance variance we found in wild species for life history traits may have profound effects on the heritability of such traits during extreme selection events such as occur in population bottlenecks.

Fisher's view that traits closely associated with fitness should exhibit low levels of additive genetic variance has been widely interpreted to mean low heritabilities (Falconer, 1981; Mousseau & Roff, 1987). This interpretation has been empirically verified (Mousseau & Roff, 1987; Roff & Mousseau,

1987). Fisher postulated that differences between heritabilities among trait categories are the result of differences in additive genetic variance, but recent theoretical considerations by Price & Schluter (1991) contest this idea. They propose that the reason life history traits have low heritabilities is because such traits are composites of many morphological, physiological and behavioural traits. Because life history traits are composites of multiple underlying traits, they are subject to numerous sources of environmental variation which in turn inflates phenotypic variance, thus decreasing the heritability. This would be the case even if the selective pressures for both life history and morphological traits were the same (Price & Schluter, 1991). To resolve this issue, one needs to examine the changes that occur in life history and morphological traits under selection for a number of generations for a large number of species. This is, at this time, an unrealistic goal. The next best thing would be to examine the relative differences that exist in the genetic components of traits for both life history and morphology traits. Although differences may exist in terms of phenotypic variance (irrespective of genetic variance) between morphological and life history traits, our data set reveals that significant differences also exist in the genetic parameters of the traits exclusive of V_p (variable D_x in Table 1a and Fig. 1). Although these results do not rule out the possibility put forth by Price & Schluter, they suggest that selection on traits closely associated with fitness will tend to change the relative contribution of additive and dominance variance in the genetic architecture of traits, and therefore lend support to Fisher's theory.

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Chapter 2. Inbreeding depression in the wild

Immediately after pronounced successional changes in a habitat or bottleneck events, the incidence of inbreeding and subsequent inbreeding depression is predicted to be high. Inbreeding depression is predicted to be greater in the wild compared to captive species due to environmental variance. This hypothesis has never been tested in the past due to the lack of enough estimates of inbreeding depression in the wild. In this chapter, I compare the mean estimates of inbreeding depression between wild populations and captive populations to test this hypothesis. This chapter has been written for publication in Heredity.

Abstract.-Although much speculation exists, despite its practical application in conservation biology and evolutionary theory, the cost of inbreeding in natural populations of plants and animals remains to a large degree unknown. In this review we have gathered estimates of inbreeding depression (δ) from the literature for wild species monitored in the field. We have also corrected estimates of δ by dividing by F (coefficient of inbreeding), to take into account the influence that the variation in F will have on δ. Our data set includes 6 bird species, 9 mammal species, 4 species of poikilotherms (snakes, fish and snails), and 15 plant species. In total we obtained 165 estimates of inbreeding depression for 134 traits of which 81 of those estimates included estimates of F. We compared our mammalian data (limited to those traits related to juvenile mortality) to the estimates for captive zoo species published by Ralls et al. (1988) to determine if, as predicted from the literature, natural estimates of δ are higher than captive estimates of δ. The mean δ (significant values only and not corrected for F) for homeotherms was 0.512; for poikilotherms, 0.201; and for plants, 0.331 (animals overall: 0.362). Levels of inbreeding depression this high in magnitude will be biologically important under natural conditions since the reduction in fitness traits will be substantial. Overall, there were no striking trends for differences among taxonomic groupings. When we limited our data set to mortality traits for mammals and corrected for F=0.25 (as is the case for the Ralls et al. data set), we found a significant difference between the two data sets, wild estimates had a substantially higher mean δ: 2.155 (captive species: 0.314). Of the 165 estimates of δ, 88 were significantly different than zero, indicating that inbred wild species measured under natural conditions frequently exhibit moderate (0.20) to high (0.51) levels of inbreeding depression in fitness traits.

Inbreeding depression is the decline in the value of a trait that is a direct consequence of inbreeding (Wright 1977; Shields 1987). The most common estimates of inbreeding depression involve traits that are closely related to fitness such as reproductive traits (eg. number of eggs laid, number of young surviving) or metric traits indirectly associated with fitness (eg. ejaculate volume, plant height). The reduction of fitness after close inbreeding can be due to a number of genetic factors: the unmasking of recessive deleterious alleles (Lande 1994; Lynch et al. 1995), increased homozygosity and/or reduced allozyme variability (Falk and Holsinger 1991; Brock and White 1992; Pray et al. 1994; Vrijenhoek 1994). Whatever the genetic mechanism, inbreeding depression is a real phenomenon that has received a substantial amount of attention in the literature (Ralls and Ballou 1983, 1986; De Bois et al. 1990; Lacy et al. 1993; see Frankham 1995a and Roff 1997 for reviews). Most of the literature concerning inbreeding depression has concentrated on domestic or captive bred wild species (Ralls and Ballou 1986; for a review see Lacy et al. 1993) due to the obvious difficulties of making estimates on wild species in nature. One of the most comprehensive data sets is that of pedigrees from zoo populations (Ralls et al. 1988). Forty captive populations belonging to thirty-eight species show an average increase in mortality of 33% for inbred matings (Ralls et al. 1988). Ralls et al. (1988, page 191) suggest that "the total costs of inbreeding in natural populations are probably considerably higher", which would make the cost of inbreeding in natural populations of considerable evolutionary consequence. The implications of high levels of inbreeding depression to population extinction are obvious (Lande 1988; Caro and Laurenson 1994; Caugley 1994). However, the degree of inbreeding depression in wild populations remains controversial (see Frankham 1995a for a discussion). The two most commonly suggested reasons why inbreeding effects in natural populations may not be significant are, 1) animals in the wild avoid as much as possible close inbreeding, and therefore do not manifest the deleterious fitness effects, and 2) even if inbreeding does occur, animals are able, either behaviourally or physiologically, to deal with the deleterious genetic effects before they are manifest on a phenotypic level, while captive species, due to the conditions of captivity, cannot respond in such a

manner. Although evidence of inbreeding depression in wild species has been published (see Frankham, 1995a for a short review), the lack of a comprehensive review across species has lead to the remaining skepticism of its existence in natural populations (Caro and Laurenson 1994).

The objective of the present study was to estimate the average inbreeding depression for wild species measured under natural conditions. We are concerned here not with whether inbreeding occurs in the wild (although we do report the coefficient of inbreeding, F, for those studies for which it was available) but rather the consequence of inbreeding on organisms living in the wild. Specifically we attempt to answer three questions: 1) is inbreeding depression in wild populations of sufficient magnitude to be biologically important should inbreeding occur? 2) does inbreeding depression differ between different groups of organisms? and, 3) does inbreeding depression differ between natural and captive populations?

Materials and Methods

1. The data set

We obtained 165 estimates of inbreeding depression from the literature. The data set includes 34 species (19 animals, 15 plants) and 134 traits (reference list available from P.C.). We included only species that were sampled from wild populations or species that were artificially inbred in the laboratory for one generation and their progeny released in the area from which their parents originated. Where more than one estimate was given for a particular trait, we included all estimates in the analysis. In addition to the two categories "animals" and "plants", we divided animals into homeotherms and poikilotherms, giving us four broad "taxonomic" groupings.

To standardize relative differences in fitness traits, we used the coefficient of inbreeding depression δ (Lande and Schemske 1985):

$$\delta = 1 - \frac{X_I}{X_O},$$

where X_I=inbred trait value and X_O=outbred trait value. To further standardize estimates of δ we changed traits such as juvenile mortality (where it is expected that $X_O < X_I$) to juvenile survivorship (so that $X_O > X_I$). This was done so that all estimates were "positive" where the *a priori* prediction is that outbred estimates should be greater than inbred estimates. Certain traits (e.g. sperm abnormalities in lions) that could not be modified since they were not expressed as portions of a total, were not used in the analysis. We included traits that were either directly related to fitness, e.g. total eggs laid, or traits indirectly related to fitness, e.g. juvenile weight.

Because the magnitude of inbreeding depression will vary with the coefficient of inbreeding (Falconer 1989), we corrected δ estimates by standardizing with respect to the coefficient of inbreeding, F. The change in trait value due to inbreeding is,

$$b = \frac{X_O - X_I}{F},$$

where b is the slope of the relationship between trait value and F. Because X_O will vary among traits, we scale the above by dividing throughout by X_O giving,

$$b = \frac{1 - \frac{X_I}{X_O}}{F}.$$

Since $1 - \frac{X_I}{X_O}$ is the measure of inbreeding depression, δ, we can simplify the equation to,

$$b = \frac{\delta}{F}.$$

Now since $\delta = bF$, the standardized slope is equivalent to inbreeding depression when F=1. Therefore dividing the estimates of δ by F, allows for a standardized comparison of the degree of inbreeding depression across taxonomic groupings while taking into consideration

different coefficients of inbreeding and trait scales. Since not all of the studies reported F values, we used only those studies that reported both δ and F estimates for this analysis. We obtained 81 estimates of F from 14 studies. We called the F corrected data sets $b-$ (includes negative values due to $X_O < X_I$) and $b+$ (negative values changed to zero).

2. Statistical analysis

All statistical analyses were done using SYSTAT (Wilkinson 1991). Because the data sets consist of ratios, we used non-parametric (Kruskal-Wallis) in addition to the parametric analyses (ANOVA). Although the data violate the conditions of parametric analysis, we have included the results when they do not differ from the non-parametric analysis to allow for post-hoc Tukey HSD tests. We divided each data set into those estimates of δ that were significant and those that were non-significant to determine how often significant levels of inbreeding depression were detected. For each estimate of δ (δ, $b-$, $b+$) parametric and non-parametric statistical analyses were conducted using either the entire data set (both significant and non-significant δ estimates--hereafter referred to as data set1) or only significant δ estimates (hereafter referred to as data set2). Differences among groups (homeotherms, poikilotherms, plants) were tested using a one-way analysis of variance. We also calculated mean values from data sets 1 & 2 for each study (producing data set3 from data set1 and data set4 from data set2) and used ANOVA to test for differences across taxonomic groups, because the over-representation of a large number of estimates from a few studies might bias the analysis of data sets 1 and 2. Because of the problem of multiple tests (4 data sets with 3 tests per set), significant differences are reported at a Bonferroni corrected P value of $\frac{0.05}{12}$=0.004.

In addition to the ANOVA test, we used the non-parametric Kruskal-Wallis test to determine if significant differences exist between estimates of δ across species categories. Data sets 1, 2, 3 and 4 for all three different δ estimates were used for the non-parametric analyses.

To determine if environmental conditions increase δ compared to captive conditions we compared mean δ values from our data set with the data set included in the Ralls et al. (1988) review of δ in captive bred

populations of wild species. We were not able to use the corrected δ data sets because there were no reported estimates of F for mammals. Ralls et al. calculated the slope of ln(survival) versus inbreeding and then predicted δ for a level of inbreeding of F=0.25. Because the Ralls et al. data set was limited to survival of offspring of mammals only, we limited our data set to traits directly related to survival in mammals. Our estimates were obtained from the δ data set and corrected for F=0.25. We used a students t-test to determine if significant differences exist between mean estimates of δ between the two data sets.

Results

1. Magnitude of δ

Theory suggests that females should not mate with their closest relatives unless the cost of inbreeding is less than 0.33 (Smith 1979). In addition, an increased probability of extinction occurs just below intermediate levels of inbreeding (Frankham 1995b). We found very high mean estimates of inbreeding depression for species measured in the wild. For δ estimates, mean inbreeding depression ranged from 0.267 to 0.512 (30% of estimates >0.33); for $b-$ estimates, mean inbreeding depression ranged from -0.843 to 0.913 (59% of estimates >0.33); and for $b+$ estimates, mean inbreeding depression ranged from 0.295 to 1.213 (59% of estimates >0.33). Most of these estimates of inbreeding depression are sufficiently high in magnitude (>0.33) to be biologically important (Smith 1979; Frankham 1995b).

2. Patterns of Variation of δ and F Across Taxonomic Groups

For δ estimates, irrespective of the data set, the pattern of variation is the same across the four taxonomic groupings; homeotherms have the highest mean δ estimates, while poikilotherms and plants have the lowest (Fig. 1). The ANOVA revealed no significant effect (significance level of P=0.004) of group on mean δ across taxonomic groups for any of the data sets (Table 1). In addition, all frequency distributions of δ for homeotherms, poikilotherms and plants for the entire data set show a strong central tendency (Fig. 2).

For the $b-$ estimates, the only significant results were for the Kruskal-Wallis analysis for data set1 (Table 1). The parametric analysis for data set1 revealed no significant effect of group.

The ANOVA analysis for $b+$ estimates revealed a significant effect of group for the entire data set (data set1; Table 1). A post hoc Tukey HSD test (Zar, 1990) showed that significant differences were found between homeotherms and plants (P=0.0001). The Kruskal-Wallis analysis revealed a significant effect of group for data set1 (Table 1).

The mean inbreeding coefficient, F, for the entire data set was 0.571±0.044. A comparison of mean F values across taxonomic grouping revealed that homeotherms (0.113±0.011) have the lowest mean inbreeding coefficient, plants (1.000±0.000) have the highest, and poikilotherms (0.328±0.021), are intermediate. Both ANOVA and Kruskal-Wallis tests revealed a significant effect of taxonomic grouping (P=0.0001) with all three groups having significantly different mean F estimates (Tukey HSD: P=0.0001).

Fig. 3 shows the frequency of significant δ estimates within taxonomic group. Chi-square analysis showed that a significant and substantial difference exists in the number of significant δ estimates for homeotherms, poikilotherms and plants (P=0.01). Because of this, the power to detect significant differences in inbreeding depression across taxonomic groupings in the above analyses may be impaired.

3. Wild and Captive Comparisons of δ

The comparison of our data set (limited to only those δ estimates of mortality of mammals and corrected for F=0.25) with that of Ralls et al. (1988) revealed a highly significant difference between δ estimates for juvenile mortality (our data set: n=9, $\bar{x}$=2.155±0.482; Ralls et al. data set: n=40, $\bar{x}$=0.314±0.044; t=7.687, df=47, P=0.0001). As predicted by Ralls et al. (1988), wild estimates of δ are substantially higher than captive estimates.

Discussion

We found that statistically significant levels of inbreeding depression in the wild are detected approximately 53% of the time when species are known to be inbred. When significant, mean inbreeding depression (not corrected for the coefficient of inbreeding, F) ranged from a low of 0.20 in poikilotherms, to a high of 0.51 in homeotherms. Therefore, species on average will experience a reduction of 20 to 50% in fitness related traits when inbred in the wild. When corrected for F, and using only positive

estimates of $b+$, mean inbreeding depression ranged from a low of 0.47 in plants, to a high of 0.88 in homeotherms. Although we found significant differences between taxonomic grouping for different estimates of inbreeding depression for certain data sets, no overall trends in significant differences were detected. The analysis using only mammals revealed a significant difference, with estimates of δ from free ranging mammals being substantially higher than estimates of δ from captive populations (2.16 and 0.31, respectively). Therefore, as predicted by Ralls et al. (1988), conditions experienced in the wild increase inbreeding depression (similar findings have been made for plants; reviewed by Roff 1997). In addition, despite the significant variation in the mean coefficient of inbreeding, F, across different taxonomic groupings, the general patterns of mean inbreeding depression (homeotherms-highest, plants-lowest) are, for the most part, maintained across the non-corrected and corrected data sets (δ, $b-$ and $b+$). Thus, the results of the non-corrected data set (δ) can be viewed as relatively unbiased by variation in F.

Although we have demonstrated that inbreeding depression under natural conditions is much higher than under captive conditions, we lack sufficient data to determine which environmental factors cause such an increase. Inbreeding depression is typically more severe in harsher environments (Falk and Holsinger 1991; Hoffmann and Parsons 1991; Latter et al. 1995; for a review see Miller 1994). Environmental factors such as unpredictable rainfall, fluctuating temperatures and limiting resources to feed young are all likely to have a significant effect on juvenile mortality. Weak inbred young that would normally die in the wild would most likely survive in captivity with veterinary assistance (Ralls et al. 1988). Some studies have shown that individuals with relatively low allozyme heterozygosity and/or with a high number of lethal equivalent alleles are much more susceptible to factors that don't seem to affect "normal" individuals (Pierce and Mitton 1982; O'Brien et al. 1985; Mitton et al. 1986; Murphy et al. 1987; Ralls et al. 1988; Fritz and Simms 1992; for examples in which no effects are observed see review in Roff 1997). Another possibility is that inbreeding depression in captivity is biased upwards due to poor husbandry practices or is an artifact of captive breeding. It has been argued that a reduction in fitness traits is to be

expected in animals that have greatly dissimilar genetic backgrounds, which from most accounts is a common occurrence in captive populations (Smith 1993); in such cases outbreeding depression may have been misdiagnosed as inbreeding depression (Templeton 1987). The ongoing debate concerning whether the seriously reduced reproductive capacity of cheetahs in captivity is due to genetic factors or incorrect captive conditions, is a good example of the difficulty of determining the cause of low fitness even for an individual species (Merola 1994; O'Brien 1994; Caro and Laurenson 1994). However, poor husbandry techniques for captive species will increase inbreeding depression, which means that in a situation where an inbred population is maintained under ideal conditions, the inbreeding depression in survival will probably be lower than the Ralls et al. estimate of 0.33 and will be substantially lower than our calculated mean of 0.539. Irrespective of the effects of poor husbandry on captive estimates of inbreeding depression, our results show that inbreeding depression in the wild will be substantially higher than in captive populations.

There are a number of important implications of high levels of inbreeding depression in wild species. Populations that experience high levels of inbreeding and subsequent inbreeding depression may in future generations have significantly lower levels of inbreeding depression even if closely inbred due to purging of deleterious recessive alleles expressed during inbreeding (Wright 1977; Lorenc 1980; Bryant et al. 1990; Barrett and Charlesworth 1991; Ribble and Millar 1992). Nevertheless, although the phenomenon of purging deleterious alleles has been documented (see Husband and Schemske 1997 for a review), studies have questioned the extent of purging (see Frankham 1995a for a discussion), and accelerated rate of inbreeding in populations can potentially drive a population towards extinction (Gilpin and Soule 1986). Although the susceptibility of most populations of animals and plants to high levels of inbreeding and inbreeding depression is poorly known, our results show that inbred organisms in the wild do exhibit inbreeding depression (frequency of significant δ=0.55) and that the levels of inbreeding depression in the wild are substantially higher than previously thought (Ralls et al. 1988). The importance of inbreeding depression for wild populations depends not only on the magnitude of

the effect when it occurs but also the likelihood of inbreeding. While high levels of inbreeding have been observed in some populations of animals and plants (Thornhill 1993; Husband and Schemske 1997) much more data are needed to ascertain its frequency.

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Table 1. Results of analysis of variance (ANOVA) and Kruskal-Wallis test of mean inbreeding depression estimates across the three taxonomic groupings (homeotherms, poikilotherms, plants) for the three estimates of δ (δ, $b-$, $b+$). Note: after Bonferroni correction, significance level for α=0.05 is 0.004.

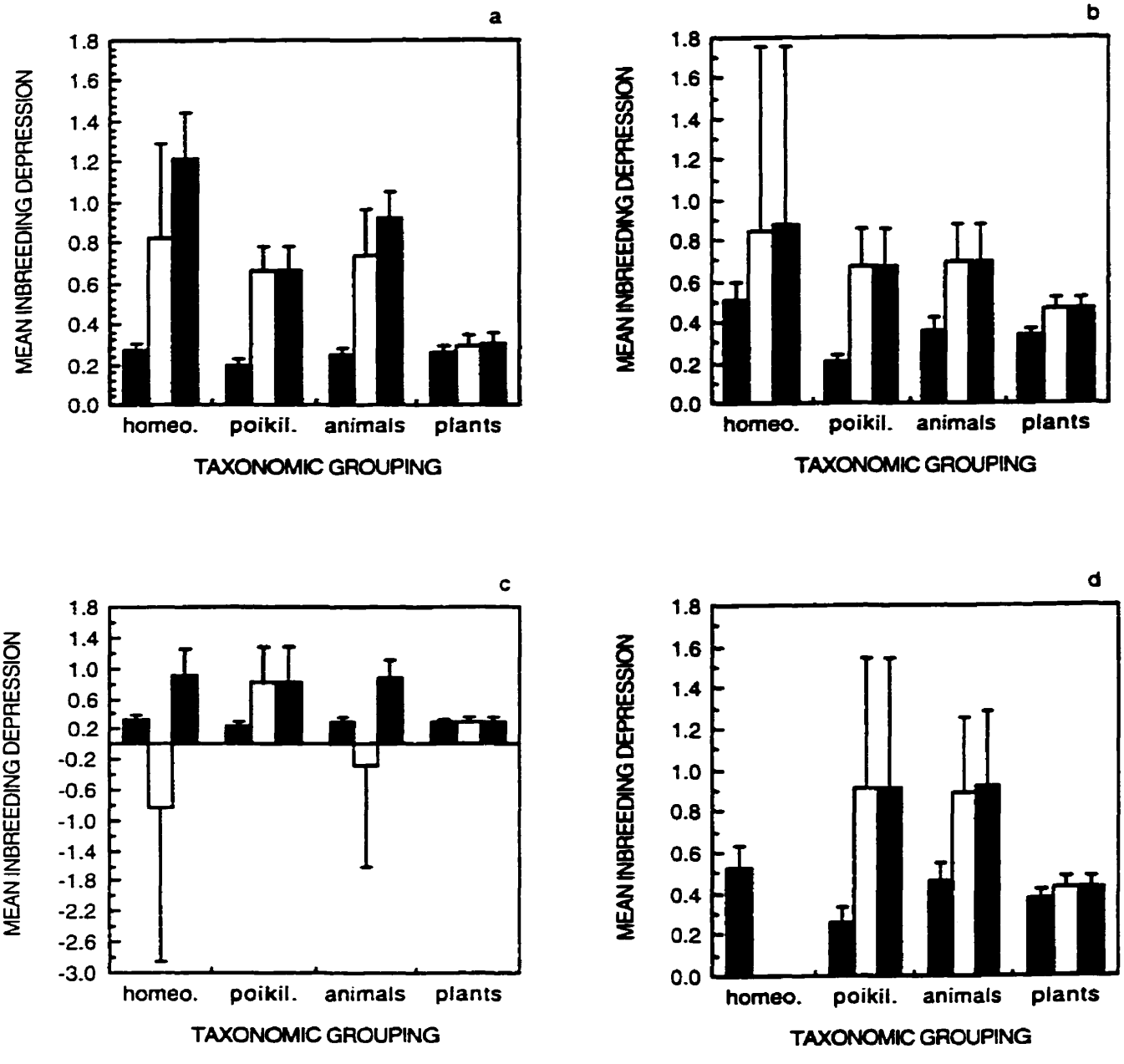
δ estimate	*n*	ANOVA *P*	Kruskal-Wallis *P*
Entire Data Set (data set1)			
δ	165	0.529	0.570
$b-$	77	0.214	0.001
$b+$	77	0.0001	0.001
Significant Estimates (data set2)			
δ	87	0.009	0.014
$b-$	36	0.377	0.997
$b+$	36	0.341	0.997
Study Means (data set3)			
δ	33	0.631	0.600
$b-$	14	0.606	0.242
$b+$	14	0.038	0.244
Significant Study Means (data set4)			
δ	28	0.202	0.466
$b-$	11	0.212	0.360
$b+$	11	0.200	0.360

Figure Captions

Fig. 1. Mean inbreeding depression (non-corrected and corrected for F) and standard error bars for the four taxonomic groupings using, a) entire data set (data set1), b) significant estimates (data set 2), c) study means (data set3), and d) significant study means (data set4). Note: solid bars=δ, white bars=$b-$(negative values included), grey bars=$\delta+$(negative values changed to zero).

Fig. 2. Frequency distributions of inbreeding depression (δ) for the entire data set (data set1). Note: a=homeotherms, b=poikilotherms, c=plants.

Fig. 3. The frequency of significant estimates of δ compared to the total number of estimates for each taxonomic grouping. Note: homeo.=homeotherms, poikil.=poikilotherms.


a
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MEAN INBREEDING DEPRESSION
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1.6
1.4
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homeo.
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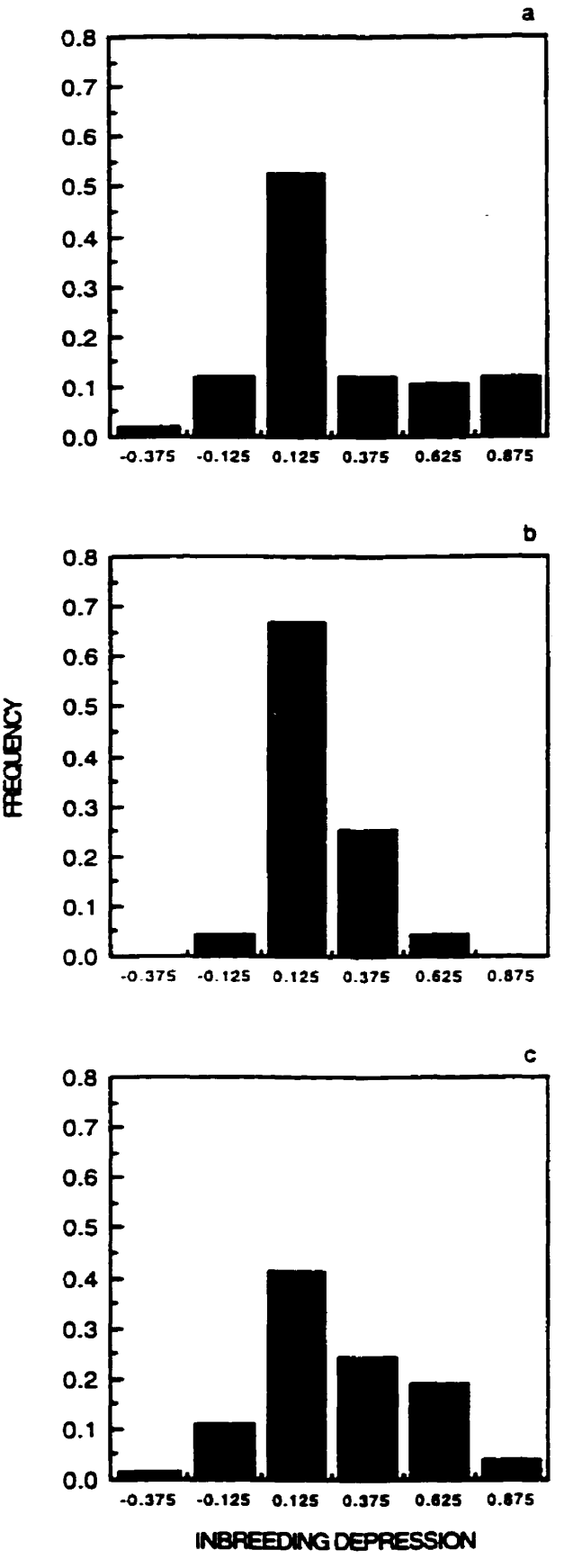
TAXONOMIC GROUPING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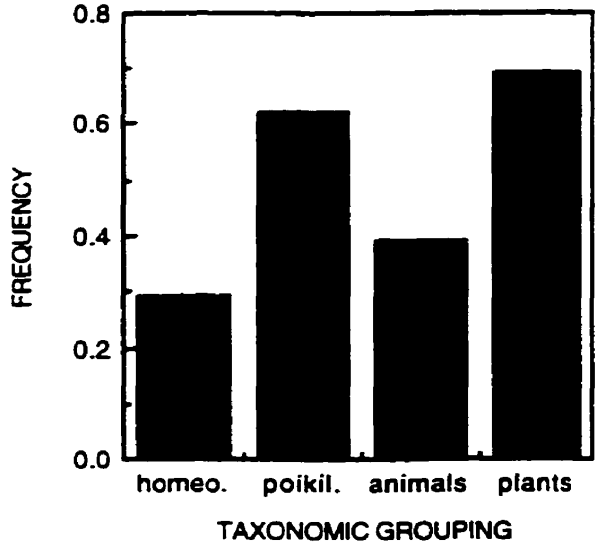
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TAXONOMIC GROUPING

Chapter 3. Fitness differences associated with calling behaviour in the two wing morphs of male sand crickets, *Gryllus firmus*.

Although the extreme selection pressures that exist during succession may cause inbreeding and subsequent inbreeding depression, insects have evolved adaptations to avoid or minimize the detrimental effects of successional changes. In this chapter I establish if a trade-off exists to macroptery in male *G. firmus*. By measuring the amount of time spent calling on day 6 of adult life for each male and his neighbour and whether or not he attracted a female, I quantify the relative time spent calling and the subsequent likelihood of attracting a female. Macropterous males are predicted to call less and therefore attract fewer females than micropterous males. This chapter has been published in Animal Behaviour (volume 50, pages 1475-1481, 1995).

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Fitness differences associated with calling behaviour in the two wing morphs of male sand crickets, *Gryllus firmus*

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Abstract. Alternate morphologies exist in a wide range of species. A commonly encountered dimorphism in insects is wing dimorphism, in which one morph is winged (macropterous=LW) and flight-capable while the other has reduced wings (micropterous=SW) and cannot fly. *Gryllus firmus* is a wing-dimorphic cricket found in the southeastern U.S.A. Although trade-offs associated with wing dimorphism are well established in female crickets, no such trade-offs have been demonstrated in male crickets. Differences between morphs in male *G. firmus* in the likelihood of attracting a female were tested in the laboratory using a simple T-maze where females chose between an LW male and an SW male. Time spent calling for each male was recorded on the sixth day of adult life. SW males were more likely to attract a female and spent more time calling than LW males. A logistic regression of female choice against the absolute proportional difference in calling time between males revealed that, as the difference in calling time between males increased, the likelihood of a female choosing the longer-calling male also increased. Therefore it is concluded that there is a trade-off between macroptery and the likelihood of attracting a female, and that it may be a primary factor in the maintenance of wing dimorphism in male *G. firmus*.

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Discrete morphologies exist in a wide range of species, and frequently play an important role in the ability to defend a territory or acquire mates. Examples of intra-sexual dimorphisms associated with such behaviour patterns include beetles (Eberhardt 1979, 1980, 1982; Brown & Bartalon 1986; Cook 1987; Goldsmith 1987; Eberhardt & Gutierrez 1991; Goldsmith & Alcock 1993), thrips (Crespi 1988a, b), fish (Dominey 1980; Gross, 1982, 1984, 1985, 1991; Maekawa & Onozato 1986; Hutchings & Myers 1987, 1988; Brantley et al. 1993), mites (Radwan 1993) and damselflies (Watanabe & Taguchi 1990). In this paper we examine the consequence of wing dimorphism on potential reproductive success in males of the sand cricket.

Wing dimorphism, in which one morph (macropterous) has long wings and possesses a functional flight apparatus and the other (micropterous) has short wings and is flightless, has independently evolved in most of the major orders of insects (Harrison 1980; Roff 1986a, 1994). The obvious advantage of being macropterous and flight-capable is the ability to move from a less favourable patch to a more favourable one, or to forage over large areas and in three-dimensional space (Roff 1990). In some cases of dimorphic variation, one morph may lead to reduced fitness and adopt the 'best of a bad lot' strategy (Eberhardt 1982); however, the wide occurrence of dimorphisms suggests that, in general, the dimorphism is maintained in the population as a consequence of trade-offs (Gross 1984; Roff 1984; Hazel et al. 1990; Roff & Fairbairn 1994). The existence of wing dimorphism suggests a trade-off between resources devoted to dispersal (wings, flight muscles and flight fuels) and those devoted to reproduction (Roff 1984, 1986a; Crespi 1988a; Denno et al. 1991; Roff & Fairbairn 1991; Zera et al. 1994).

Among wing-dimorphic insects, trade-offs between macroptery and reproductive potential have been well studied in females. Although no consistent pattern of adult longevity or development time has been documented between morphs, differences have been found in age at first reproduction and overall fecundity. Micropterous females reproduce earlier and have a higher

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fecundity than macropterous females (Roff 1986b; Denno et al. 1989; Roff & Fairbairn 1991; Mole & Zera 1993).

Although the existence of negative phenotypic correlations between wing morph and reproduction has been well documented in female insects, few examples of such general trade-offs have been found in male insects. Male *Hoplothrips karnyi* have a foreleg dimorphism that is correlated with the wing dimorphism, wingless males having long forelegs and winged males short forelegs (Crespi 1988a). Males with longer forelegs win fights more often and became dominant at oviposition sites (Crespi 1988a). Utida (1972) cited anecdotal evidence that in the coleopteran *Callosobruchus maculatus*, the most frequent copulations occurred between wingless males and females. Brachypterous (short-winged) males of the planthopper *Nilaparvata lugens* develop earlier and outcompete macropterous males (Novotny, in press). In *Aquarius remigis*, wingless males were found mating more often than winged males (Kaitala & Dingle 1993). Fujisaki (1992) has shown that a fitness advantage exists to wing reduction in male *Cavelerius saccharivorus* in terms of earlier sexual maturity, leading to a greater proportion of matings in favour of the brachypterous morph.

No general trade-offs for wing dimorphism in males have been found in *G. firmus*. Roff & Fairbairn (1994) found no difference in testes size or the proportion of offspring sired between morphs of *G. firmus*, although macropterous males develop earlier than micropterous males. This result is contrary to the proposed trade-off hypothesis, and therefore cannot account for the preservation of the two morphs in males (Roff & Fairbairn 1994). Despite the fact that no general costs to macroptery were found in male *G. firmus*, an overview of the distribution of the frequency of macroptery between the sexes in wing-dimorphic insects suggests a potential trade-off in the Orthoptera. In most insect orders composed of species that are wing-dimorphic, there is either no difference between the sexes in proportion macroptery (Coleoptera) or males have a slightly higher proportion of macroptery (Homoptera and Hemiptera). In the Orthoptera (grasshoppers and crickets), on the other hand, proportion macroptery is substantially higher in females than in males, suggesting that macroptery in males may be more costly (Roff & Fairbairn 1994).

The Orthoptera differ from other orders in that mate attraction involves calling by the males. Calling is an energetically demanding behaviour requiring on average a 10-fold increase in metabolic rate over the resting rate (data for seven species from Table 1 of Bailey et al. 1993). In the genus *Gryllus* and in other orthopterans, the likelihood of attracting a mate is directly proportional to time spent calling (Butlin et al. 1985; Hedrick 1986; Cade & Cade 1992; Tuckerman et al. 1993; cf. Zuk 1987). It is not known whether energetic resources needed for wing production, flight muscle formation and the maintenance of both, constrain resources needed for calling. If such a constraint exists, then a trade-off between macroptery and calling may promote the maintenance of the two morphs in male *G. firmus*.

In the earlier experiments of Roff & Fairbairn (1994) the males and females were kept in close proximity (in a 4-litre bucket) and hence costs associated with calling would not have been manifested. The experiment reported here tests the hypothesis that a trade-off results from the calling behaviour of the two morphs. Specifically we test the hypothesis that, in *G. firmus*, micropterous males attract more females than macropterous males. We hypothesize that such a difference is a consequence of time spent calling. If the energy needed to form and maintain the flight apparatus competes with that needed for calling in macropterous males, we predict that micropterous males should call longer during a 24-h period than macropterous males.

METHODS

Species Description and Methods of Rearing

Gryllus firmus is a large (approximately 0.75 g), ground-dwelling cricket commonly found in the southeastern U.S.A. and as far north as Connecticut (Alexander 1968; Harrison 1985). It is usually found in early successional and sandy areas, which are likely to favour the evolution of wing dimorphism (Roff 1990, 1994). Subjects originated from a colony founded from approximately 40 individuals (20 males, 20 females) from a locality in northern Florida (Roff 1986a). Stock crickets were maintained in the laboratory at approximately 100–300 breeding adults at a temperature of 25–30°C for approximately 36 generations before being used in this experiment. Subjects were maintained as nymphs in disposable

mouse cages (29 × 19 × 13 cm) at a constant temperature of 28°C and a 15:9 h light:dark photoperiod with ad libitum food and water. After 1 week in the mouse cages, we placed nymphs in circular buckets (21 × 15 cm) at a density of 60 per bucket and maintained them in the same incubators set at the same temperature and photoperiod as before. Food consisted of crushed Purina rabbit chow provided ad libitum. Water was provided by a soaked cheesecloth wick connected to a water reservoir in a second bucket into which the first bucket was suspended. On the day of their final ecdysis (the day nymphs became adults), we placed males in individual buckets containing ad libitum food (pellets, not crushed as before) and access to water. We maintained males in these personal buckets until they were needed for the experiments. Under such rearing conditions all crickets were exposed to the same environmental conditions (photoperiod, temperature and acoustical experience). We also placed females in individual buckets, and maintained them under the same environmental and food conditions as males until 6 or 7 days old, when we then used them in the experiment.

Experimental Design

Six days following final eclosion, we placed each male in an individual glass jar (9 cm in diameter) with ad libitum food and water. We used crickets that were 6 days old because preliminary analysis indicated that by this day call duration levels off and remains constant thereafter (personal observation; comparative data from three *Gryllus* and one *Teleogryllus* species: Cade & Wyatt 1984). We placed each glass jar in a bucket which was connected to two other buckets by plastic tubing, 2.5 cm in diameter (see Fig. 1), thereby forming a T-maze. We concurrently used eight T-mazes (two mazes per incubator), all of which were placed in incubators set at 15:9 h L:D and 28°C. In each T-maze, we placed two males (one micropterous=SW, one macropterous=LW) and a female (either LW or SW, chosen at random) in separate buckets (Fig. 1). The tubing that inter-connected all three buckets allowed the females easy and free access to a male. Cones placed on the end of each tube leading to a male's quarters prevented the female from exiting the male's bucket that she first entered. The jars housing the males were painted black to prevent

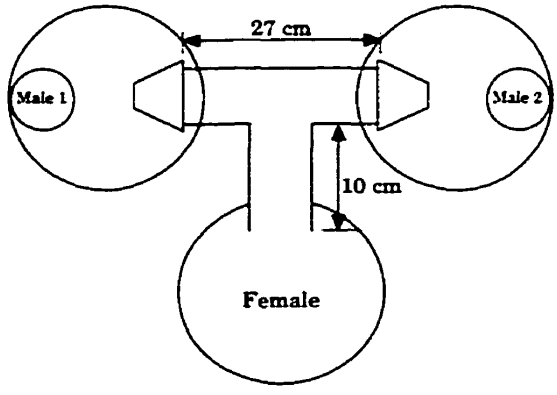

Figure 1. Top view of the T-maze used in the call monitoring/female selection experiment.

the male and female from seeing each other. Wire mesh covered the top of each jar to allow females to hear calling males. We used a continuous playback of cricket calling to provide a constant background in each incubator because male crickets tend to call more often when they hear others call (Cade & Wyatt 1984).

We monitored male calling using a Realistic tie-clip 33-105 microphone (frequency response, 50–15 000 Hz) placed in each male's jar. Microphones were connected to an analogue-to-digital converter relay system monitored by a computer that recorded the time of each incoming signal. The gain of each microphone was set at a level that would trigger the relay system only when the occupant of the jar called and not by the background call or the calls of neighbouring crickets. Every male was monitored for 23.5 h. We cleaned each T-maze after a pair of males was monitored in order to minimize the possibility that females moving through a maze were influenced by olfactory cues (or faeces) left by previous females. To the best of our knowledge no research has documented the existence of olfactory cues released by males to attract females in *G. firmus*. In addition, Roff & Fairbairn (1994) found no differences between male wing morphs with respect to the success at siring offspring when males and females are kept within close proximity, which is indirect evidence that no morph-specific olfactory cues exist. We monitored 47 males. Because of the lack of appropriate pairs, three T-mazes at one time contained only single (SW) males.

Statistical Analysis

We assessed female choice using a goodness-of-fit test, with the null hypothesis that the probability of female attraction should be equal for each morph in the male pair. Because the a priori hypothesis was that SW males should attract more females than LW males, we used a one-tailed test (critical $\chi^2=2.71$).

We used a Student's t-test to assess whether significant differences existed in total time spent calling for the 23.5 h that we monitored males. Because we predicted a priori that SW males call longer than LW males, we used a one-tailed test.

We assessed the effect of call duration on female attraction using the dependent variable, Y (female choice for the longer or shorter calling male: 0=shorter, 1=longer), and the independent variables C (relative call rate),

$$\frac{|SW-LW|}{SW+LW}$$

and F (female choice for a particular wing morph: 0-LW, 1-SW). The basic assumption of normality underlying regression analysis is violated because the data consist of 0, 1 values (Sokal & Rohlf 1981). Therefore, we used a logistic regression (SAS 1988) in addition to the multiple linear regression. Because the results of the two tests did not differ, for simplicity, those of the linear regression are also presented.

RESULTS

Female Attraction

We introduced 22 females, one in each T-maze, of which 20 females chose a male in the LW/SW male pair. Only females that made a choice were used in the subsequent analysis. Of the 20 females used in the analysis, 15 moved into a SW male's quarters and five moved to a LW male's quarters. As predicted, SW males attracted a female significantly more often than did LW males ($\chi^2=5.00$, $df=1$, $P<0.01$).

Call Duration

We monitored 22 pairs and three single SW males. The microphone of one LW male malfunctioned, resulting in 21 LW males used in the analysis. The mean ($\pm$ SE) call duration for SW males was 1.09 ± 0.14 h. The mean call duration for LW males was 0.60 ± 0.11 h. SW males had significantly longer call durations than did LW males ($t=-2.653$, $df=44$, $P<0.01$).

Regression Analysis

The overall regression for both variables (C and F) was highly significant (linear: $r^2=0.400$, $N=19$, $P<0.017$). The likelihood of a female moving to the longer calling male increased with the relative call rate, C (logistic: $df=1$, $P<0.025$; linear: $r^2=0.178$, $df=1$, $P<0.020$). Even after accounting for effects due to relative call rate, females preferred SW males over LW males, F (logistic: $df=1$, $P<0.035$; linear: $r^2=0.222$, $df=1$, $P<0.005$).

DISCUSSION

Flight capability, although advantageous to crickets because it permits them to cope with changing conditions such as successional changes in their habitat, is not without costs. Producing and maintaining the long wings that macropterous crickets possess is probably not energetically costly, but the production and maintenance of the massive flight muscles is energetically expensive (Roff 1989; Mole & Zera 1993; Tanaka 1993). Because of this energetic constraint, we predicted that micropterous males would call longer and attract more females than macropterous males. The present study supports this hypothesis. The level of calling in micropterous males is comparable to that found for other species of *Gryllus* crickets in the wild (Cade & Cade 1992). We are presently conducting studies where males are monitored for 3 weeks to obtain a detailed record of call behaviour and female attraction. These studies will also determine if other factors of call behaviour, such as bout duration, in addition to total time spent calling influence female attraction. In an analysis of song components of *G. firmus*, Webb & Roff (1992) found no difference between morphs in pulses/chirp, pulse rate, chirp length or frequency, but did observe a significantly smaller pulse length in micropterous males (23.3 ± 0.3 versus 26.1 ± 0.4 ms, SW/LW, respectively). However, the variability in pulse length (SD approximately 4.3) is such that it appears unlikely that a female could use the trait as a means of reliably distinguishing between morphs.

Female *G. firmus* appeared to base their choice for a particular male in part on the duration that the male spent calling. A regression of female choice on the proportion of time spent calling revealed that males that called more than their neighbour increased their chance of attracting a female. Analysis also revealed, however, that females preferred micropterous males even after accounting for effects due to relative call rate. This result suggests that other factors of calling may be involved that give micropterous males an additional advantage over macropterous males. Other parameters of calling behaviour, such as sound intensity or call bout length, may be involved in the attraction of a female (Doherty & Hoy 1985; Hedrick 1988; for a review see Tuckerman et al. 1993), but because the amount of energy required to sustain calling is very high, call duration is likely to be the most important parameter with respect to the energetic trade-off hypothesis.

Roff & Fairbairn (1994) hypothesized that the evolution and maintenance of wing dimorphism in *G. firmus* is a function of (1) fitness differences between morphs and (2) genetic correlations of wing form between the sexes. Roff & Fairbairn (1994) have shown that the inter-sexual genetic correlation of wing form in *G. firmus* is quite high (0.86), which is in accordance with other estimates of the genetic correlation of morphological traits between the sexes (Price & Burley 1993; review in Roff & Fairbairn 1994). If the genetic correlation is exactly ± 1, a change of the trait in one sex will be entirely dependent on the change in the other (Via & Lande 1985). Because the inter-sexual genetic correlation for wing form in *G. firmus* is less than 1, trade-offs associated with macroptery and traits associated with fitness will allow selection to eventually drive the proportion macropterous for each sex to their respective optima. Thus, the maintenance of macroptery in male *G. firmus* may primarily depend on the trade-off between flight capability and female attraction.

The present measurements of the trade-off between macroptery and the likelihood of attracting a female in male *G. firmus* demonstrate only a phenotypic basis for the trade-off. Any discussion of how trade-offs between traits can constrain evolution must also address the genetic basis of the trade-off (Reznick 1985; Stearns 1989). Without an underlying genetic basis for the traits involved, the observed phenotypic trade-offs cannot lead to a selective change from one generation to the next. Although numerous studies have demonstrated a fitness advantage to wing reduction in insects, in only one has such an advantage been shown to be genetically based. Roff (1990, 1994) demonstrated that in female *G. firmus*, the trade-offs between macroptery and fitness components such as fecundity are genetically based. Selection for decreasing and increasing incidence of macroptery was accompanied by an increase and decrease in fecundity levels of female crickets. The heritability of wing form in *G. firmus* is quite high (h^2=0.65; Roff 1986a, b); thus selection acting on this trait will rapidly lead to changes in the incidence of macroptery in a population (Roff 1992). Although the heritability of call behaviour is not presently known for *G. firmus*, Hedrick (1988) demonstrated that call bout length has a highly significant heritability (h^2=0.75) in a congeneric species, *G. integer*. Cade (1981) demonstrated that nightly mean call time in *G. integer* also has a significant heritability (approximately, h^2=0.50). The next step is to determine if there is additive genetic variance in *G. firmus* for call behaviour and if the correlation between wing form and calling is genetically based.

Although a number of studies have documented the existence of alternate morphologies within a sex, we still do not completely understand how these morphologies are maintained in a population. For example, studies of dimorphic beetles demonstrate fitness advantages of large body size and disproportionately large weaponry (horns). None the less, the advantages and disadvantages associated with a particular morphology have yet to be quantified for most of the cited examples. The two studies that have thus far examined whether fitness differences exist in wing-dimorphic crickets (Holtmeier & Zera 1993; Roff & Fairbairn 1994) have shown no differences between the morphs. In both cases, however, males and females were kept in close proximity and hence calling by the male would not have been a factor in mating success. In addition to wing morph, other sources of variation (sex ratio, population density, parasite load and environmental factors) are known to affect calling behaviour in a number of *Gryllus* species (Cade 1984; French & Cade 1987; Cade & Cade 1992; Souroukis et al. 1992; Souroukis & Cade 1993). It is not known, however, whether wing morphs are differentially affected by such sources of variation, and therefore the importance of the above

discussed factors to the trade-off hypothesis is also not known. Our study demonstrates that fitness advantages exist to microptery in male *G. firmus* with respect to the likelihood of attracting a female, and also that alternate morphs have different calling behaviour patterns. Further work is required to determine whether other differences exist between wing morphs that might lead to differences in mating ability, or whether our findings are applicable to other members of the Orthoptera, and to other insect species in general where males attract females by calling.

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Chapter 4. The contingency of fitness: an analysis of food restriction on the macroptery-reproduction trade-off in crickets.

A reproduction trade-off to macroptery exists in LW males, under the conditions of *ad libitum* food and constant environmental conditions in the laboratory. The question of whether the trade-off is ecologically important in the wild depends upon the amount of resources typically available. This question can be resolved by considering the two extremes-*ad libitum* and maintenance levels. In this chapter I use crickets that have been energetically stressed (by restricting food intake) to determine if, as hypothesized, the magnitude of the trade-off will increase. Stressed crickets are predicted to call less and therefore attract fewer females than *ad libitum* fed crickets. This chapter has been published in Animal Behaviour (volume 50, 1998).

The contingency of fitness: an analysis of food restriction on the macroptery-reproduction trade-off in crickets

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We examined the effect of food limitation on fitness trade-offs between macroptery and time spent calling in the wing-dimorphic cricket, Gryllus firmus. The results of previous studies have shown that, under optimal conditions, the proportionate time that males spend calling (with respect to neighbouring males) is directly associated with female attraction, and that micropterous (short-winged: SW) males call longer than macropterous (long-winged: LW) males (Crnokrak & Roff 1995, Animal Behaviour, 50, 1475–1481). Because crickets were examined under optimal conditions, these studies did not attempt to address how or whether fitness trade-offs change with the environment. In the present study, crickets received ad libitum water but we restricted food intake to the minimum amount that would keep them alive for 20 days. On average, SW males called longer than LW males on 18 of 20 days in the stressed group, and 17 of 20 days in the control group. For both groups, SW males also attracted more females more often than did LW males. Although the absolute call durations decreased in the stressed group compared with the control group, the relative call durations remained approximately the same, as did the proportion of females moving towards the SW male. Cumulative call distributions showed that LW males called little after 10 days of adult life; the amount of time SW males spent calling seemed constant for the duration of the experiment.

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Trade-offs are a central facet of life history evolution (e.g. Stearns 1977, 1989; Reznick 1985; Pease & Bull 1988). Although phenotypic trade-offs are well documented (e.g. Stearns 1989), some studies have shown no significant trade-offs, others have shown positive associations between traits (Garland 1988; Brodie 1989, 1991, 1993; Jayne & Bennett 1990). Phenotypic trade-offs based on physiological constraint of resources will probably be sensitive to the environmental conditions under which they are measured; that is, a trade-off may be manifested under resource-limited conditions but not under conditions of resource abundance. Experiments that involve the artificial manipulation of traits are more successful at measuring trade-offs than experiments in which the association of individual traits are simply recorded under laboratory conditions (Bell & Koufopanou 1986; van Noordwijk & de Jong 1986; Tatar & Carey 1995). Thus, one should expect to observe quantitative (magnitude changes) and sometimes qualitative (directional changes) variation in trade-offs with changes in the environment.

The effect of environmental variance on trade-offs has been commonly examined in trade-offs that involve traits affected by physiological resource partitioning. Evidence for a shift towards more negative (less positive) covariances for trade-offs involving offspring size and offspring number, present and future reproduction, and development rate and fecundity in more stressful environments has been found in zooplankton (Spitze 1995). The trade-off between mortality and reproduction in *Callosobruchus* beetles is lowered by 12% when food is available at the peak of egg production (Tatar & Carey 1995). Variation in temperature affects trade-offs between morphology and development in butterflies so much that the sign of the correlation between the traits changes (Windig 1994).

The effect of environmental variation on behavioural trade-offs has been studied less often. A trade-off between territorial defence and survivorship was observed in the lizard *Sceloporus jarrovi* only when subjects were injected with testosterone (Marler et al. 1995). In two spider species, *Nephila claviceps* and *N. maculata*, the negative correlation between weight gain and the size of the web at high food levels changed to a positive correlation at lower food levels, presumably because spiders avoid weight loss by not investing in unnecessary web synthesis when food is abundant (Higgins 1995). Thus, variation in resources can strongly affect behavioural trade-offs.

For trade-offs to affect the evolution of the component traits, they must (1) have a genetic basis (heritable component in the independent traits and a negative genetic

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correlation between the traits) and (2) be prevalent under conditions that would normally be encountered in the wild (Stearns 1989). Studies conducted under novel conditions in a laboratory setting may show that a trade-off exists, but may not indicate that it is biologically relevant. Trade-offs that involve a differential allocation of energetic resources are sensitive to variation in such variables as the amount and rate of resources acquired, the efficiency with which such resources are used and the variation of resources distributed to different functions (e.g. Mole & Zera 1994, in press; Zera et al. 1994). Many behavioural trade-offs are also context and condition dependent (reviewed in Boake 1994). In dimorphic species of insects, trade-offs often involve behaviours associated with a dimorphism that is used in territory defence and mate acquisition (reviewed in Crnokrak & Roff 1995). To address the general importance of such constraints on the independent evolution of the traits involved, it is necessary to measure trade-offs in different environments to determine how these constraints change with the environment.

Trade-offs have been extensively examined in insect species with dimorphisms for wing length: macropterous individuals have long wings and can usually fly, whereas micropterous individuals have short wings and cannot fly (reviewed in Roff 1986; Crnokrak & Roff 1995). Wing dimorphism is maintained by the balance between the advantages accruing to migrants (macropters) in a variable environment and the disadvantages of reduced fecundity in migrant females (Roff 1994). Macropterous males appear to be less successful than micropterous males at obtaining mates (reviewed in Crnokrak & Roff 1995). In female sand crickets, *G. firmus*, a phenotypic trade-off exists between macroptery and the age schedule of reproduction (measured as time to reproduction and cumulative fecundity; see Roff & Fairbairn 1994). On the physiological level, the trade-off is a consequence of an energetic constraint that limits the production and maintenance of both the flight apparatus and eggs (*G. firmus*: Roff 1989; Mole & Zera 1994, in press; *G. rubens*: Mole & Zera 1993; *Modicogryllus confirmatus*: Tanaka 1993). The traits involved in the trade-off (wing morph, fecundity) have a genetic basis and are genetically correlated in *G. firmus* (Roff 1990, 1994), and in another cricket, *Allonemobius socius* (Roff & Bradford 1996). In *G. firmus* (as in many species of Orthopterans), males are relatively sedentary as adults and attract females for mating by producing a species-specific calling song (e.g. Alexander 1968; Cade & Cade 1992). Calling is energetically expensive, requiring on average a 10-fold increase in metabolic rate (Forrest 1991b; Bailey et al. 1993). Studies on Orthopterans have shown that the likelihood of attracting a female increases with time spent calling (e.g. Butlin et al. 1985; Forrest 1991a; Tuckerman et al. 1993; but see Zuk 1987). Trade-offs in male *G. firmus* have been found between macroptery and time spent calling (Crnokrak & Roff 1995); over a 24-h period, macropterous males called less and attracted fewer females than micropterous males. We hypothesized that the trade-off for males is mediated, as it is for females, by competition for restricted resources between the flight apparatus and calling. Although the trade-off exists on a phenotypic level, it is not known whether it changes with age or, if and how, it is affected by variations in resource availability.

In this study, we examined trade-offs between macroptery and calling behaviour in *G. firmus* to address two main questions. (1) Is the trade-off maintained in male crickets during a large portion of their adult lives? Crnokrak & Roff (1995) monitored males for only 1 day of their adult lives (day 6). The present study provides a more detailed account of age-specific trade-offs between macroptery and time spent calling. (2) How is the trade-off affected when crickets are subjected to a resource stress? Although other factors such as photoperiod and temperature may also affect calling, we predicted that the amount of food available to crickets would have the most pronounced effects, because we hypothesized that the behaviour associated with this trade-off is determined by an energetic constraint. If the trade-off exists under both optimal and extreme suboptimal conditions, we would conclude that the costs would be found in free-ranging, wild populations.

METHODS

Experimental Design

Gryllus firmus is a relatively large (0.7 g) cricket common on successional sandy areas along southeastern U.S.A. and as far north as Connecticut (Alexander 1968; Harrison 1985). Subjects were taken from a breeding stock originally started from approximately 20 males and 20 females captured in northern Florida (Roff 1986). Stock crickets were maintained in the laboratory as a breeding group of around 300 adults for about 38 generations before being used in this experiment. Subjects were maintained as nymphs in mouse cages (29 × 19 × 13 cm) at a constant temperature of 28°C and a 15:9 h light:dark photoperiod with ad libitum water and food. After 1 week in the mouse cages, we placed nymphs in circular buckets (width 21 cm; height 15 cm) at a density of 60 per bucket and placed them in the same incubators set at the same temperature and photoperiod as before. Under such conditions all crickets were exposed to the same conditions (photoperiod, temperature and acoustical experience). We fed crickets crushed Purina rabbit chow provided ad libitum. Water was provided to the crickets by a soaked cheese cloth wick connected to a water reservoir in a second bucket into which the first bucket was suspended. We placed adult females in individual buckets and maintained them under the same environmental and food conditions as males until they were 6 or 7 days old, at which time we used them in the experiment.

Once they were adults, we placed each male in an individual glass jar (9 cm in diameter) with the appropriate amount of food (see below for description) and ad libitum water. We placed each glass jar in a separate bucket, which was connected to two other buckets by plastic plumbing tubing, 2.5 cm in diameter (Fig. 1), thereby forming a T-maze. We used eight T-mazes concurrently (two mazes per incubator), placed in incubators set at 15:9 h L:D and 28°C. Each T-maze contained two

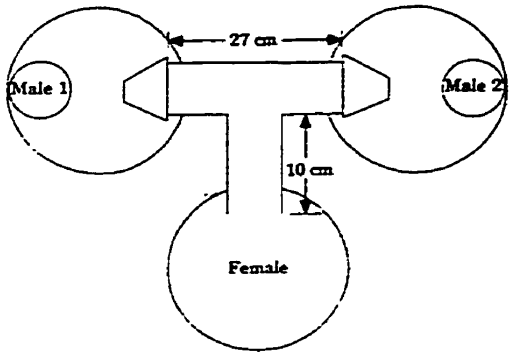

Figure 1. Top view of the T-maze used to monitor female choice between two calling males. Each T-maze contained one micropterous (SW) and one macropterous (LW) male and a female (LW or SW, at random).

males, one micropterous(SW), one macropterous(LW) and a female (either LW or SW, chosen at random) in separate buckets (Fig. 1). The tubing that interconnected all three buckets in a T-maze allowed the females easy and free access to each male. Cones placed on the ends of each tube leading to a male's quarters prevented the female from leaving the male's bucket that she first entered. The jars housing the males were painted black to prevent the male and female from seeing each other. Wire mesh was glued to the top of each jar to allow females to hear calling males. We used a continuous playback of cricket calling to provide a constant background in each incubator, because male crickets tend to call more often when they hear others call (Cade & Wyatt 1984).

We monitored male calling using a Realistic tie-clip 33-105 microphone (frequency response=50–15 000 Hz) placed in each male's jar. Microphones were connected to an analog-to-digital converter relay system monitored by a computer that recorded the time of each incoming signal. The gain of each microphone was set at a level that would trigger the relay system only when the occupant of the jar called, and thus was not triggered by the background call or the calls of neighbouring crickets. Field studies on rangeland grasshoppers have shown that mortality is about 5% per day (Onsager & Hewitt 1982); thus after 20 days, 95% of individuals in a population are dead. We therefore monitored crickets for 20 days posteclosion (unless a male died prematurely; see Results for sample sizes). When one male in a pair died, we no longer introduced females into that T-maze but still monitored the remaining male for time spent calling. We used 15 male pairs in the stressed group and six pairs in the control group, which received ad libitum food and water (the reduced sample size was due to space and time limitations). We cleaned each T-maze every day after monitoring each pair of males to minimize the possibility that females moving through a maze were influenced by olfactory cues (or faeces) left by previous females. In addition, Roff & Fairbairn (1994) found no differences between male wing-morphs in success at siring offspring when males and females were kept in close proximity, suggesting that no morph-specific olfactory cues exist.

To determine the smallest amount of food needed daily to keep the crickets alive for 20 days, a preliminary study was undertaken in which groups of crickets were fed different amounts of food. To minimize effects of body size, we chose individuals that were near the average weight (~0.75 g). A food level of 0.01 g of crushed rabbit chow per day was the lowest amount of food necessary to keep crickets of average weight alive for 3 weeks. Although no physiological or phenotypic analyses were conducted on the crickets used in the preliminary experiment, we assumed that feeding crickets the lowest amount of food necessary to keep them alive constituted a notable energetic stress. For practical considerations, we fed the male crickets in the stressed group 0.03 g of food every third day starting on the day of their final moult. This level of food restriction is comparable to the study by Tanaka (1993) of energetic constraints between fecundity and macroptery in the cricket *M. confirmatus*.

Statistical Analysis

We assessed female choice for both treatment groups (stressed and control) using a goodness-of-fit test with the null hypothesis that the probability of attracting a female should be equal for each morph in the male pair. Because the a priori hypothesis was that SW males should attract more females than LW males, we used a one-tailed χ^2 test (critical χ^2=2.71). We assessed overall significance for both groups with two tests; first, a χ^2 test of the number of pairs in which the SW male attracted the female more often than the LW male; second, a t test using, p, the proportion of times within each pair the SW male attracted the female (arcsine square-root transformed). In the second case, the null model is that p=0.5 (p=0.45 when arcsine square-root transformed).

A Student's t test was used to test for differences in total time spent calling for each morph for the 20 days they were monitored (separate t tests for stressed and control groups). Because a priori, SW males are predicted to call longer than LW males, we used a one-tailed test. To determine the effect of age on 'lifetime' calling patterns, we plotted cumulative daily means and medians for SW and LW crickets for the 20 days they were monitored, and daily mean and median call durations for both groups.

The effect of call duration and male morph type on female attraction was assessed using the following model.

$$Y=\text{constant}+C+F+PR+C*F,$$

where, Y is female choice for the longer or shorter calling male (0=shorter, 1=longer), $C=|SW-LW|/SW+LW$ (relative call rate), F is female choice for a particular wing morph (0=LW, 1=SW), and PR is the pair designation (categorical variable, N=15 stressed group; N=6 control group). From this model, we predicted that, for both groups, as the relative call rate C increases (greater difference in calling between paired males), females should choose the longer-calling male, Y=1. Because we predicted that SW males would be the longer-calling male

Table 1. Total number of females attracted by micropterous (SW) and macropterous (LW) males placed in each T-maze

Pairs	Number of females attracted to male		Total number of females	Female preference
	LW	SW		SW>LW
Stressed				
1	5	11	16	Yes
2	4*	8*	12	Yes
3	6*	5	11	No
4	2	16	18	Yes**
5	5	11	16	Yes
6	2*	8*	10	Yes**
7	0	4*	Yes**	
8	2	13	15	Yes**
9	0*	8	8	Yes**
10	4	9	13	Yes
11	3* 5	8	Yes	
12	7	6	13	No
13	2*	3	5	Yes
14	0	6*	6	Yes**
15	3	10	13	Yes**
Control				
1	6	10	16	Yes
2	4	8	12	Yes
3	4	6	10	Yes
4	3	10	13	Yes**
5	5	12	17	Yes**
6	5	6	11	Yes

Total=the total number of females per T-maze that made a choice for either male. Because SW males were predicted to attract more females than LW males, the goodness-of-fit test was one-tailed (χ^2=2.71, not Bonferonii corrected). *Indicates which crickets died prematurely. **Indicates that the SW male attracted significantly more females than did the LW male (P<0.01).

more often than LW males, the relationship between Y and F should also be positive. The regression of Y on F also allowed us to test for a morph effect on female attraction when C was held constant. We used the variable PR to test the effect of pair on Y because of the potential non-independence of each data point; because we monitored each male for 20 days, the regression of Y on F and C includes repeated measures. Because the data consist of the values 0 and 1, the basic assumption of normality underlying regression analysis is violated (Sokal & Rohlf 1981), so we used a logistic regression (SAS 1988) in addition to the multiple linear regression.

Despite our efforts to ensure that stressed crickets survived the full 20 days of the experiment, some SW and LW crickets died prematurely (no control crickets died prematurely). To test for differences in average longevity between the morphs for those males that died (six LW, four SW) we used an independent samples t test. Because a priori we expected LW males to be more energetically stressed than SW males and therefore more likely to die sooner, the t test was one-tailed.

RESULTS

Female Attraction

The goodness-of-fit test results showed that in seven of the 15 pairs of stressed males there was a significant bias in favour of SW males with respect to the total number of females attracted; in two of the six control pairs there was a significant bias in favour of SW males (Table 1). SW males attracted more females more often than did LW males in 13 of the 15 pairs of stressed males (χ^2_1=8.07, P< 0.002); all of the control pairs showed a bias in favour of SW males (χ^2_1=6.00, P<0.003). For both groups, SW males had a higher probability of attracting a female than LW males (stressed: p=0.79, t=4.36, P< 0.0001; control: p=0.66, t=6.803, P<0.0001; p=probability after back transformation). There was no significant difference between control and stressed groups in the probability of attracting a female (t_{19}=1.34, P=0.20).

Call Duration

In assessing total time spent calling between the wing morphs, we used only those males that survived the full 20 days (stressed group: 11 SW and 9 LW; control group: 6 SW and 6 LW). The mean ± SE 'lifetime' call durations for stressed crickets were 9.34 ± 0.96 h (SW) and 4.57 ± 0.90 h (LW); for control crickets they were 24.51 ± 3.00 h (SW) and 14.28 ± 4.26 h (LW). Both treatment (control and stressed) and morph type (SW and LW) had significant effects on total call duration (treatment: F_1 ratio=50.353, P=0.0001; morph: F_1 ratio=11.550, P=0.002). The relative time spent calling (SW/LW) was

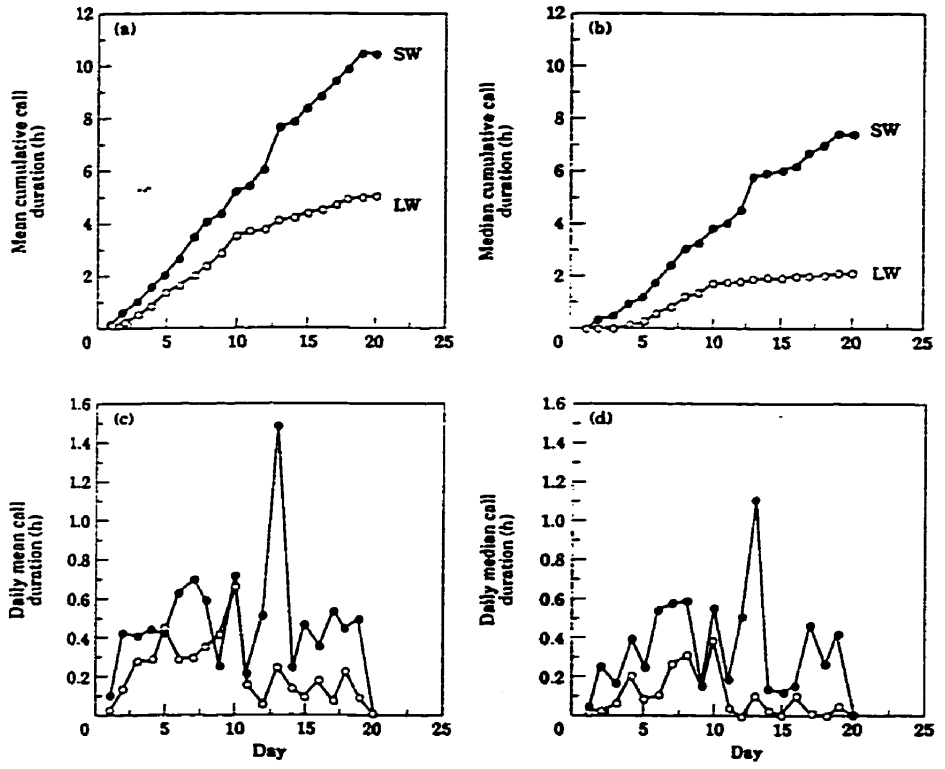

Figure 2. Cumulative (a, b) and daily (c, d) mean and median call durations for stressed crickets; ●: micropterous; ○: macropterous.

similar for both groups (control group: 1.71; stressed group: 2.04).

For the stressed group, there was a divergence between SW and LW males in daily time spent calling after day 10; LW males called much less on average after day 10 than they did previously (cumulative line for LW males levels and daily values were relatively lower than SW values; Fig. 2), but SW males continued to call approximately the same amount of time before and after day 10 (cumulative line for SW males was almost linear and greater than LW daily values; Fig. 2). Similar call patterns were also found in the control group, except that the divergence between morphs was not as pronounced as in the stressed group (Fig. 3). Cumulative medians and daily median values for the stressed group (Fig. 2) revealed similar effects, except that the divergence between morphs after day 10 was even greater. The use of median values in place of means compensates for the effects of a few long-calling individuals on the average call duration for any one day. Because the means and median graphs were similar, we concluded that no one male (either SW or LW) influenced the patterns of divergence in the means graphs. SW males also called longer than LW males (mean daily values) on 18 of the 20 days of monitoring (Fig. 2).

Female Attraction and Call Duration

Because the results of the logistic regression did not differ from the linear regression (for both groups), for simplicity, we present only the results of the latter analysis. We found a significant interaction between morph and call duration for both treatment groups (Table 2). Because of the significant interaction between *C* and *F*, and the nonsignificant *PR* term, we further analysed the data using the following model.

$$Y = \text{constant} + C,$$

With the data set divided according to the morph preference of the female; subset 1(*SWPREF*) included only those data points in which the female chose the SW male, and subset 2(*LWPREF*) included only those data points in which the female chose the LW male. The likelihood of a female moving to the longer-calling male increased with the relative call rate in the *SWPREF* subset, but for the *LWPREF* subset, relative call duration had no effect on female choice for the stressed group (Table 2). However, call duration had a significant effect on female choice for both subsets in the control group (Table 2). Although the

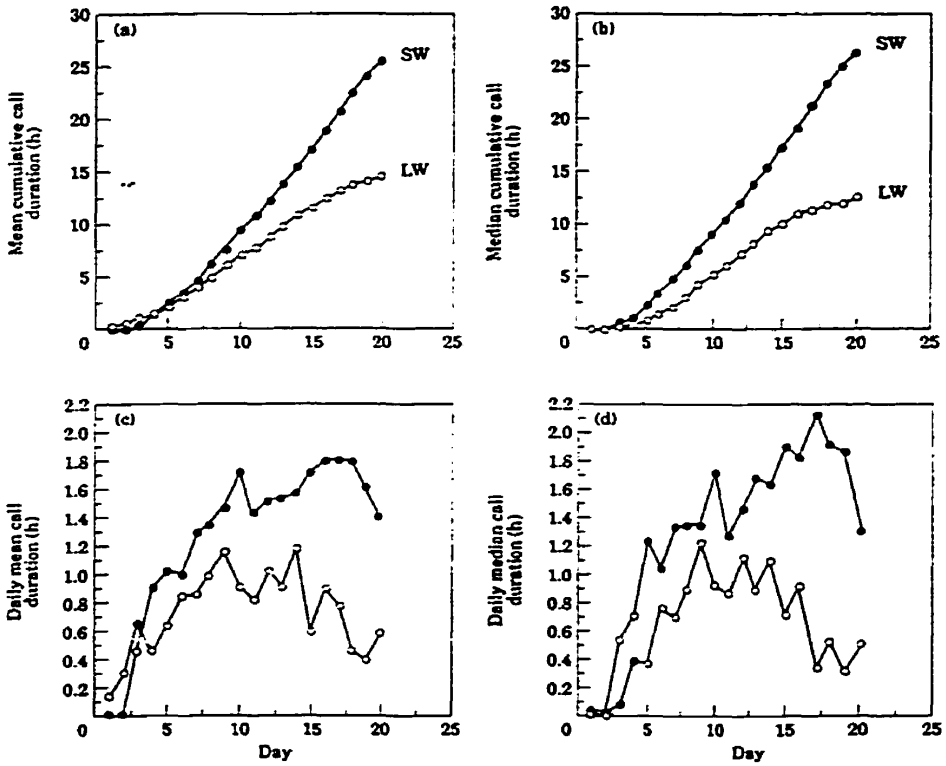

Figure 3. Cumulative (a, b) and daily (c, d) mean and median call durations for control crickets; ●: micropterous; ○: macropterous.

Table 2. ANOVA and regression analysis for models 1 and 2 for stressed and control groups (analysed separately)

Source	Stressed crickets			Control crickets		
Model 1	*df*	F	P	df	F	P
C	1	5.161	0.025	1	0.955	0.332
F	1	2.095	0.150	1	8.872	0.004
PR	14	1.036	0.422	5	0.433	0.817
*C*F*	1	10.907	0.001	1	11.474	0.001
Model 2	*N*	r	P	N	r	P
LWPREF						
C	39	0.089	0.5912	27	0.348	0.075
SWPREF						
C	110	0.493	0.0001	51	0.427	0.002

Where C equals | SW−LW | /SW+LW, *F* denotes female choice for a particular wing morph (0: LW, 1: SW) and *PR* denotes pair designation (15 in total). *LWPREF* includes only those data points where the female chose the LW male, and *SWPREF* includes only those data points where the female chose the SW male.

use of the second model on the two subsets did not allow us to test for female preference of male morph type, females chose SW males more often than LW males in both treatment groups, even when relative call rate was not taken into consideration (Table 2).

Longevity

Of those crickets that died prematurely, the average life span was 12.8 ± 1.7 days for SW males and 13.7 ± 1.4 days for LW males (t_n=0.414, P<0.69).

DISCUSSION

Flight capability, although obviously advantagous in certain circumstances, is not without significant costs. Producing and maintaining the flight muscles is energetically expensive for macropterous crickets (e.g. Mole & Zera 1993, 1994, in press; Zera et al. 1995). Calling is also energetically costly, given that metabolic rates can increase up to 10-fold during bouts of calling (Bailey et al. 1993). Because resources for muscle maintenance are hypothesized to restrict resources available for calling, this energetic constraint is predicted to be manifested as a behavioural constraint. A phenotypic trade-off exists between macroptery and calling behaviour in *G. firmus*; macropterous males call less than micropterous males and subsequently attract fewer females (Crnokrak & Roff 1995). The present study has shown that this phenotypic trade-off was maintained for a large portion of an adult cricket's life; the greatest differences occurred when crickets were in their breeding prime (10–20 days). Food limitation reduced the total amount of time males spent calling in the stressed group compared with the control group, but the relative total time spent calling (SW/LW), and thus the probability that SW males would attract more females was similar for both groups. With respect to the absolute changes, comparable effects of food restriction have been documented in *Requena verticalis*. Male *R. verticalis* provided with a high-quality diet allocate 57% of their daily available energy to calling but allocate only 30% to calling when provided a poor-quality diet (Simmons et al. 1992). The present study demonstrates the effect of resource limitation on a trade-off that involves morphology and behavioural mate acquisition.

The maintenance of wing dimorphism in male *G. firmus* depends upon the trade-off between mate acquisition and flight capability, which as demonstrated in the present study can exist under conditions of resource abundance and severe resource limitation. Provided that all individuals are subject to the same resource regime, the relative fitness of macropterous and micropterous males, with respect to the probability of attracting a mate, remains the same. If there is variation in the amount of resources acquired by males, however, there may be either a decrease or increase in the relative fitnesses. For example, a micropterous male that obtains only minimal rations will call for about the same duration as a well-fed macropterous male. Hence, we hypothesize that both males will have the same probability of attracting a female. Thus, although the mean relative fitness may be the same in an environment with variability in resource acquisition as in a homogeneous environment, there will be an increase in the variance in fitness and a consequent reduction in the strength of selection. This finding is important because similar trade-offs may be widespread among dimorphic species in which the dimorphism is involved in, or associated with, the defence of a territory and/or the acquisition of mates (reviewed in Roff 1996).

Although resource restriction is probably the most important factor affecting calling behaviour, it is not the only one. Field and laboratory studies have shown that other environmental and social factors can significantly affect the calling behaviour of a cricket. In *G. fultoni*, pulse duration, pulse period and chirp period decreased with increasing temperature, although no quantitative analysis was conducted (Doherty & Callos 1991). Pulse period and pulse duration have also been shown to decrease with increasing temperature in *G. bimaculatus* (Doherty 1985). Similar temperature effects have been shown in other crickets (*Oecanthus* sp.; *Anurogryllus* sp. (Walker 1962a, b, 1963; Prestwich & Walker 1981). The time of day and photoperiod have measurable effects on calling behaviour: *G. integer*, *G. veletis* and *G. pennsylvanicus* males tend to call most at sunset and just before dawn (French & Cade 1987). *Gryllus integer*, *G. bimaculatus* and *G. campestris* (Simmons 1986; Hissmann 1990; Cade & Wyatt 1984; Cade & Cade 1992) and the grasshopper *Ligurotettix coquilletti* (Greenfield & Shelly 1985) decrease calling at high densities, presumably because they shift their behaviour to searching for mates and defending territories (Cade & Wyatt 1984). Similarly, as the sex ratio of females increases, call duration decreases (Souroukis & Cade 1993). Finally, selection by acoustically orienting flies may affect the calling behaviour of crickets (Cade 1991; Cade & Wyatt 1984). Reduced calling in *G. integer* and *G. rubens* is hypothesized to be the result of parasitoid flies present in wild populations (Cade 1991). What is not known however, is how different wing morphs are affected by these environmental and social factors, and whether the receiver of the signal (female) is also affected in the same manner as the signaller.

The ultimate goal of studying trade-offs in dimorphic species is to explain how such constraints mediate the evolution of the individual traits involved. Such an investigation requires that the constraints be measured on a phenotypic, physiological and genetic level (Reznick 1985; Stearns 1989). Phenotypic analyses are most common, but physiological and genetic analyses lag far behind (Pease & Bull 1988; Stearns 1989). Although this study went beyond simply measuring the trade-off between wing morph and time spent calling, further analysis is needed to answer how the intermediary link between genetics and phenotype operates. Trade-offs between macroptery and egg production seen on a phenotypic level are determined by a trade-off of resources at the physiological level for females of *G. rubens* (Mole & Zera 1993), *Teleogryllus oceanicus* (Roff 1989), *M. confirmatus* (Tanaka 1993) and *G. firmus* (e.g. Roff 1989; Zera et al. 1995). No physiological studies on male crickets have been conducted to determine how resources are allocated between the flight apparatus (wings, flight muscles and flight fuels) and time spent calling. We are presently conducting lipid and wing muscle analyses on different groups of crickets across different ages and correlating changes in these traits with changes in calling behaviour to answer this question. We have also just completed breeding studies that establish a genetic basis to wing morph and time spent calling, and a genetic correlation between the two traits. In light of the genetic analysis, the present study indicates that the evolutionarily important trade-off (as defined by Reznick 1985) between wing morph and time spent calling also occurs under conditions encountered in the wild and may be a means

by which the wing dimorphism is maintained in male *G. firmus*.

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Chapter 5. The genetic basis of the trade-off between calling and wing morph in males of the cricket, *Gryllus firmus*.

The trade-off between macroptery and the likelihood of attracting a female that I have shown exists on a phenotypic level will be of no consequence to the evolution of the traits involved if those traits have no genetic basis and if there is no genetic correlation between them. In this chapter, I have examined the genetic basis of the trade-off by estimating the heritabilities for call duration, wing morph and flight muscle weight using a full-sib breeding design. In addition, I have estimated the genetic correlations between call duration and wing morph and call duration and flight muscle weight. This chapter is published in Evolution (Volume 52(4), 1998).

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THE GENETIC BASIS OF THE TRADE-OFF BETWEEN CALLING AND WING MORPH IN MALES OF THE CRICKET, *GRYLLUS FIRMUS*

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Abstract.—Wing dimorphisms exist in a wide range of insects. In wing-dimorphic species one morph is winged, has functional flight muscles (LW), and is flight-capable, whereas the other has reduced wings (SW) and cannot fly. The evolution and maintenance of wing dimorphisms is believed to be due to trade-offs between flight capability and fitness-related traits. Although there are well-established phenotypic trade-offs associated with wing dimorphism in female insects, there only exist two studies that have established a genetic basis to these trade-offs. The present study provides the first evidence for a genetically based trade-off in male insects, specifically in the sand cricket *Gryllus firmus*. Because they have to expend energy to maintain the flight apparatus (especially flight muscles), LW males are predicted to call less and therefore to attract fewer females. To be of evolutionary significance, call duration, wing morph, and wing muscle condition (size and functionality) should all have measurable heritabilities and all be genetically correlated. Differences between morphs in male *G. firmus* in the likelihood of attracting a female were tested in the laboratory using a T-maze where females chose between a LW male and a SW male. Call duration for each male was recorded on the sixth day of adult life. A significant difference in call duration was found between SW and LW males (SW = 0.86 ± 0.01, LW = 0.64 ± 0.01 h). SW males attracted significantly more females than did LW males (63% vs. to 37%). All the traits involved in the trade-off had significant heritabilities (call = 0.75 ± 0.33; wing morph = 0.22 ± 0.07; muscle weight = 0.38 ± 0.09) and genetic correlations (call and wing morph = −0.46 ± 0.20 for SW, −0.68 ± 0.16 for LW; LW call and muscle weight = −0.80 ± 0.14). These results provide the first documented evidence that trade-offs between a dimorphic trait and a fitness-related character in males has a genetic basis and hence can be of evolutionary significance.

Key words.—Dimorphism, genetic correlation, heritability, trade-offs.

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Discrete morphological variation is found in a wide range of species. In many cases dimorphisms in body structure are also associated with behavioral modifications that play an important role in the ability to defend a territory or acquire mates (for a review in animals in general, see Crnokrak and Roff 1995). A particular type of dimorphism within the Insecta is that of wing dimorphism in which one morph (macropterous) possess a functional flight apparatus and the other (micropterous) is flightless. Wing dimorphism has evolved independently in most of the major orders of insects (Harrison 1980; Roff 1986a, 1994a). Although 99.9% of all insects belong to the "winged" superorder, Pterygota, many genera are composed of species that are dimorphic for wing length or monomorphically wingless (for reviews, see Thayer 1992; Roff 1994a).

The advantage to being flight capable is the ability to move over large areas and in three dimensional space (Roff 1990a). However, although producing and maintaining the long wings that macropters possess is probably not energetically significant, the production and maintenance of the massive flight muscles is energetically expensive (Roff 1989; Mole and Zera 1993; Tanaka 1993). Although in some cases of dimorphic variation one morph may have a reduced fitness and be adopting the "best of a bad lot" strategy (Eberhardt 1982; but see Roff 1996), the wide occurrence of dimorphisms suggests that, in general, the dimorphism is maintained in the population as a consequence of trade-offs (Gross 1984; Roff 1984; Hazel et al. 1990; Roff and Fairbairn 1993; Roff 1996). Wing dimorphisms appear to be maintained by a trade-off between resources devoted to dispersal (wings, flight muscles, and flight fuels) and those devoted to reproduction (Roff 1984; Denno et al. 1991; Roff and Fairbairn 1991; Zera and Denno 1997). In many species of macropteorus insects, the flight apparatus is not maintained throughout adult life (wings may be removed and flight muscles histolyzed), and therefore the trade-off may be important only during the time over which the flight apparatus is functional.

Most of the studies of the costs of macroptery in wing-dimorphic insects have been done using females. In general, micropterous females reproduce earlier and have a higher fecundity than macropterous females (Roff 1986a; Denno et al. 1989; Roff and Fairbairn 1991). These differences are very large in the sand cricket, *Gryllus firmus*: micropterous females reproduce significantly earlier after the final moult (two days) and produce 60% more eggs in a six-week period than do macropterous females (Roff 1984). Physiological studies have shown that fecundity differences in *G. firmus* females are due to the allocation of resources to reproduction in the SW (micropterous) morph and those to flight apparatus maintenance in the LW (macropterous) morph (Mole and Zera 1994a,b).

For a trade-off to affect the evolution of wing dimorphism, there must be a genetic correlation between the traits involved, that is, between wing morph and reproduction. Without a genetic correlation, selection for an increase in proportion macroptery will not be accompanied by a decrease in fitness-related traits and will, therefore, result in a monomorphic winged population. Genetic correlations between wing morph and fecundity have been demonstrated in female *G. firmus* (Roff 1990b, 1994c) and the cricket *Allonemobius socius* (Roff and Bradford 1996; Roff et al. 1997), indicating that the trade-off will modulate the evolution of these two traits.

Although the existence of costs to macroptery are well

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TABLE 1. Examples of components of call parameters in the Orthoptera found to be important in attracting females.

Call component	Species	Reference
Call duration	*Gryllus integer*	French and Cade 1987 Cade and Cade 1992
	Neonemobius sp.	Forrest 1991a
	Gryllus campestris	Hissmann 1990 Walker 1993 Simmons 1988
	Cyphoderris strepitans	Snedden and Sakaluk 1992
	Gryllus bimaculatus	Bohm et al. 1991
	L. coquilletti	Greenford and Shelley 1985
	Omocestus viridulus	Eiriksson 1994
	Chorthippus brunneus	Butlin et al. 1985
	Gryllus firmus	Crnokrak and Roff 1995, in press
Song intensity	*Scapteriscus* sp.	Forrest 1983
	Gryllus integer	Cade and Cade 1992
	Gryllus pennsylvanicus	Cicerán and Murray 1994
Call bout length	*Gryllus integer*	Hedrick 1986, 1994 Hedrick and Dill 1993
Vibratory signals	*Gryllus bimaculatus*	Weidmann and Keuper 1987
Syllables per phrase	*Scudderia curvicauda*	Tuckerman et al. 1993
Pulse structure	*Acrididae* sp.	von Helversen and von Helversen 1983

documented in female insects, only a few examples of costs have been studied in male insects. Phenotypic trade-offs to macroptery have been shown to exist in thrips, coleopterans, planthoppers, waterstriders, and a hemipteran (Crnokrak and Roff 1995; Fairbairn and Preziosi 1997). The above-mentioned studies have demonstrated phenotypic trade-offs between morphological and life-history traits in male insects, but to date no study has examined the genetic basis of these trade-offs.

In *G. firmus*, as with many other Orthopterans, males are relatively sedentary as adults (Alexander 1968; Dadour 1990; Cade and Cade 1992) and attract females by calling. A number of components of calling are known to be important cues for female attraction, of which one of the most important appears to be total time spent calling (Table 1). Studies on Orthopterans have shown that the likelihood of attracting a female is typically proportional to call duration (for references, see Table 1). Calling is energetically demanding, requiring on average, a 10-fold increase in metabolic rate (data for seven species from Table 1 of Bailey et al. [1993] and one species from Forrest 1991b). A phenotypic trade-off has been demonstrated in male *G. firmus*, with micropterous males calling longer and attracting more females than macropterous males (Crnokrak and Roff 1995, in press). Wing form in *G. firmus* has a significant and high heritability (h^2 = 0.65; Roff 1986b). In *G. integer*, call bout length has a significant and high heritability (0.75; Hedrick 1988, 1994), whereas Cade (1981) found in the same species that mean nightly time spent calling also has a significant heritability (0.50). These data suggest that there will be additive genetic variance for call duration in *G. firmus*, and that a genetic correlation between wing morph and call duration is feasable (i.e., both h^2 are high). Without a genetic correlation between them, the evolution of wing dimorphism and call duration will not be jointly constrained. A significant negative genetic correlation between calling and wing morph would mean that selection acting on one trait would result in a correlated response of the other, a necessary requirement for any trade-off to be evolutionarily important (Stearns 1989; Roff 1994c).

In the present study we examined the genetic basis of the trade-off between wing morph and calling behavior in *G. firmus*, addressing two main questions: (1) are the traits involved in the trade-off (call duration, wing morph and flight muscle condition—a measure of the degree of histolysis) significantly heritable? and (2) is there a significant genetic correlation between call duration and wing morph and call duration and flight muscle condition?

MATERIALS AND METHODS

Species Description and Methods of Rearing

Gryllus firmus is a large (live weight, ≈ 0.75 g), ground-dwelling cricket found in the American Southeast as far north as Connecticut (Alexander 1968; Harrison 1985). It is usually found in early successional and sandy areas, which being impermanent favor the evolution of wing dimorphism (Roff 1990a, 1994b). Individuals in this experiment originated from approximately 40 individuals (20 males, 20 females) from a locality in northern Florida (Roff 1986b). Stock crickets were maintained in the laboratory at approximately 100–300 breeding adults at a temperature of 25–30°C for approximately 38 generations before being used for the behavioral experiments. Crickets used for the experiments were maintained as nymphs in disposable mouse cages (29 × 19 × 13 cm) in incubators set at a photoperiod of 15 h light, 9 h dark and at a temperature of 28°C with ad libitum food and water. After one week in the mouse cages, nymphs were placed in 4-L buckets (diameter = 21 cm; height = 15 cm) at a density of 60 nymphs per bucket. Food consisted of crushed Purina rabbit chow provided ad libitum. Water was provided by a soaked cheesecloth wick connected to a water reservoir in a second bucket into which the first bucket was suspended. On the day of their final ecdysis (the day nymphs became adults), individuals where removed from these rearing buckets and

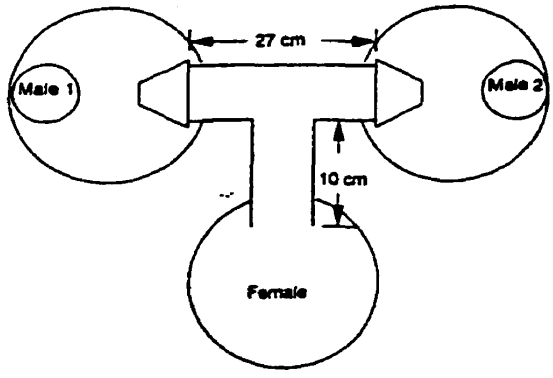

FIG. 1. Schematic diagram of the T-maze.

placed in individual quarters containing food and water until they were six days old, at which time they were used in the experiment.

Experimental Design

General Setup.—Figure 1 shows the T-maze used for the behavioral experiment. Each male was housed in an individual glass jar and monitored for call duration and whether they attracted a female. Each glass jar was placed in a bucket that was connected to two other buckets by plastic 2.5-cm diameter tubing, which thereby formed a T-maze. Eight T-mazes were used, all of which were placed in incubators set at a 15:19 L:D photoperiod and 28°C. For each T-maze, two males (one micropterous [SW], one macropterous [LW]) and a single female (either LW or SW, chosen at random) were placed in separate buckets as indicated in Figure 1. The 2.5-cm tubing that interconnected all three buckets allowed the females free access to the males' quarters. Cones placed on the end of each tube leading to a male's quarters prevented the female from exiting the bucket of the male she first entered. The jars housing the males were painted black to prevent the male and female from seeing each other. Wire mesh covered the top of each jar allowing females to hear the calling males. A continuous playback of cricket calling was used to provide a constant background (Cade and Wyatt 1984).

Male calling was monitored by Realistic tie-clip 33-105 microphone (frequency response, 50–15,000 Hz) placed in each male's jar. Microphones were connected to an analog-to-digital converter relay system monitored by a computer that recorded the time of each incoming signal. The computer scanned the relay system once every second. Chirp length averages 154 msec (Webb and Roff 1992) and are repeated in bout lengths generally exceeding one second (average bout length = 1.47s; PC, pers. obs.). Each microphone gain was set at a level that would trigger the relay system only when the occupant of the jar called and would not be triggered by the background call or the call of neighboring crickets. A simple binary code was used by the computer to record when a male called (1 = calling, 0 = not calling). Every male was monitored for 23.5 h, on day 6 as adults. Day 6 was picked as the best one-day comprimise because calling increases up to day 6 and then changes little afterward (Crnokrak and Roff, in press). Recording began at 1300 h each day and ended at 1230 h the next day (30 min was spent on maintaining the food and water levels and cleaning the T-maze). After daily maintenance was completed, the computer was reset and monitoring recommenced.

Breeding Design.—Sires were obtained from eggs laid in earth dishes from the general breeding stock of crickets (see above for description). The earth dishes were placed in disposable mouse cages in incubators set at 15:9 L:D and 28°C. Nymphs were reared in 4-L buckets (60 in each) and maintained under the same photoperiod and temperature conditions as the eggs. Once adult, the males were placed in individual quarters until six days old, at which time they were monitored for call duration and female attraction. Eleven SW and 11 LW sires were monitored (sires were paired with males that were not used for the genetic experiment; sample size for sires = 22). After measurement of call durations, the sires were placed in individual buckets and allowed to mate with a female (morph picked at random) for approximately one week. Females oviposited their eggs in soil in Styrofoam cups during this time, which were incubated under the same environmental conditions as before. These nymphs (F_1 generation) were maintained in 4-L buckets until adults, at which time they were raised individually until six days old and monitored for time spent calling and female attraction. From each family, five SW and five LW male offspring were monitored (thus the total sample size for the female choice experiment was 22 sire pairs + 10 × 11 offspring = 132). The remaining crickets from each family were raised until adulthood to obtain an estimate of proportion macroptery for each family. All male crickets used in the experiment were preserved by freezing for muscle analysis.

Muscle Condition Analysis.—Preserved sires and offspring were thawed and dissected to determine weight and condition of their dorsal longitudinal flight muscle. The dorsal longitudinal flight muscles (DLMs) are situated immediately ventral to the dorsal portion of the mesothorax (Pfau and Koch 1994). Muscle condition was determined visually by comparing muscle color on a three-point scale: beige white (0), pale pink (1), and brick red (2). Muscle color is an indicator of functional (red) or nonfunctional (white) flight muscles (Ready and Josephson 1982). All color tests were performed by one person (PC) to eliminate any variation in vision perception among experimenters. Muscles were then dissected out of the crickets and dried for one week at 60°C and weighed to an accuracy of 0.0001 g. Although all male crickets were dissected, only LW individuals had measurable flight muscles.

Statistical Analysis

Descriptive Statistics.—We used a *t*-test to determine if significant differences exist between morphs in call duration (both sires and offspring) for the 23.5 h of monitoring. Because the a priori prediction is that SW males will call longer than LW males, the *t*-test was one-tailed.

We assessed female choice using a goodness-of-fit test: because the prediction is that SW males will attract more females than LW males, the test was one-tailed (critical $\chi^2 = 2.71$).

To examine the relationship between call duration and the probability of attracting a female we used the model:

$$P = b\frac{C_{SW}}{C_{SW} + C_{LW}} = bC. \quad (1)$$

where P is the probability of a female choosing the SW male, C_{SW} is the call duration of the SW male, C_{LW} is the call duraiton of the LW male, and b is a constant. If the probability of attracting a female is proportional to the relative call duration (C), then b = 1. If, as suggested by previous analyses (Crnokrak and Roff 1995) females show a bias for SW males then b > 1. Because the maximum value of P is one, if the bias is sufficiently great the relationship may be curvilinear with an asymptote at one. No such curvature was found in the present analysis and hence we shall restrict our attention to the above model. Because single females were used in each trial, P is a binary variable taking values of zero (female chose the LW male) and one (female chose the SW male). Such data do not fulfill the assumptions of least-squares regression and, therefore, we used a maximum-likelihood approach. The likelihood, L, for a sample in which the number of SW and LW males chosen is n_1 and n_0, respectively, is:

$$L = \prod_{i=1}^{n1} bC_i \prod_{j=1}^{n0} (1 - bC_j). \quad (2)$$

The slope, b, was estimated as that which maximized the above likelihood. To provide a test of the slope we used the 95% confidence interval estimated using Wald's method (Wilkinson 1996, p. 452). In addition, the above model was fitted using least squares by minimizing the mean square error.

We used ANOVA to determine if differences exists between DLM weight among muscle color groups. Because the a priori prediction is that functional muscles (group 2) will be larger than nonfunctional muscles (group 0), the test was one-tailed.

Heritability Estimates.—Full-sib heritability estimates were calculated using nested (cages nested within families) ANOVA and the jackknife (Simons and Roff 1996). As both estimates gave almost identical results we present only those from the ANOVA. Heritability estimates calculated were: time spent calling (SW and LW separate, and SW + LW), wing morph (using the threshold model, for details, see Roff 1997), and DLM weight of macropterous males. Full-sib estimates of heritability include (Becker 1995):

$$\frac{Va + \frac{1}{2}Vd + \frac{1}{2}Vi}{Vp}. \quad (3)$$

where V_a = additive genetic variance, V_d = dominance variance, and Vi=epistatic variance. Cage effects were nonsignificant and, therefore, cages were combined.

In addition to the full-sib estimates, we calculated offspring-parent heritability estimates for call duration. Because these estimates include only V_a in the numerator, a comparison of full-sib and parent-offspring heritability estimates allows us to determine the contribution of nonadditive effects to the total genetic variance. The offspring-parent heritability estimate for call duration was calculated using a regression of mean offspring values on mean sire values; where the dependent variable, offspring call duration = (SW mean call per family) × (proportion SW in family) + (LW mean call per family) × (proportion LW in family); and the independent variable = sire call duration. In addition to the above analysis we also ran the regression separately for SW and LW males (mean SW offspring call duration regressed on SW sire call duration, etc).

Phenotypic Correlation Estimates.—We calculated phenotypic correlations for call duration and wing morph and; LW call duration and DLM weight. Correlations were calculated using offspring values for both estimates. Because family sizes were equal, phenotypic correlations were calculated using a general linear regression of call duration on wing morph and DLM weight.

Genetic Correlation Estimates.—We calculated genetic correlations for LW call duration and wing morph; SW call duration and wing morph; LW call duration and DLM weight; and call duration between morphs. Because a fixed number of LW and SW offspring per family (irrespective of proportion macroptery in the family) were measured, genetic correlations could not be estimated in the usual manner (Roff and Bradford 1996). However, genetic correlations from full-sib data can be estimated, at least approximately, by the Pearson product moment correlation between family means (Via 1984; Roff and Preziosi 1994). Mean values per family were calculated by first calculating mean values per cage (as described above) and then averaging across cages.

Standard errors values for the correlation estimates were calculated using (Becker 1995):

$$SE = \sqrt{\frac{1 - r^2}{n - 2}}. \quad (4)$$

Results

Phenotypic Relationships

Call Duration.—SW males had significantly longer call durations than LW males ($t = -11.404$, df = 216, $P < 0.0001$); SW males called for 0.86 ± 0.01 h and LW males for 0.64 ± 0.01 h.

Female Attraction.—SW males attracted 83 of 131 (63%: one female did not move reducing the total sample size by one) females that made a choice for a male in the T-mazes, whereas LW males attracted 48 of the 131 (37%) females (total females that moved = 110 [offspring tests] + 21 [sire tests-1 female that did not move]). As predicted, SW males attracted significantly more females than LW males ($\chi^2 = 4.68$, $P < 0.01$).

Call Duration and Female Attraction.—There was a significant effect of relative call duration on the probability of attracting a female (95% confidence interval excluded 0; least-squares estimation gave $r = 0.83$, $P < 0.1 \times 10^{-6}$): because the SW male called longer than the LW male, the females chose the SW male more frequently. The slope for the model was significantly greater than one ($\delta = 1.17$; 95% confidence interval: 1.05–1.28; by least-squares confidence

TABLE 2. Summary of heritability estimates using the full-sib and parent-offspring analysis.

Trait	$h^2 \pm SE$	n	P
Full-sib estimates			
SW call duration	0.68 ± 0.21	110	0.002
LW call duration	0.81 ± 0.21	109[a]	0.002
SW + LW call	0.75 ± 0.33	219	0.04
Wing morph	0.22 ± 0.07	44[b]	0.01
DLM weight	0.38 ± 0.09	109	0.007
Offspring-parent estimates			
SW call duration	0.61 ± 0.39	11	0.151
LW call duration	1.07 ± 0.34	11	0.012
SW + LW call	0.72 ± 0.13	22	0.0001

[a,b] Note: sample sizes change definition depending on trait type; all of the full-sib estimates are individual values except for wing morph, which is mean proportion macroptery per cage (two cages per family); offspring-parent estimates for SW and LW call duration are regressions of mean offspring values on sire values (SW offspring on SW sires and LW offspring on LW sires); SW + LW call duration is a regression of mean offspring values on sire values for both morphs. Where [a] = 110 − 1 (one LW male died during experiment), [b] = 22 families × 2 cages per family = 44.

interval: 1.01–1.27), indicating an effect of male morph type in addition to relative call duration on the probability of attracting a female. Although there is a difference between the predicted slope value of one and the calculated value of 1.17, it is not substantial, indicating that relative call duration is much more important to female choice than male morph type (i.e., substituting a C-value of 0.57 (mean relative call duration for all crickets) into the model gives $P = 0.67$, indicating that the relative effect of male morph type is 0.10 compared to the relative call duration effect of 0.57).

DLM Weight.—The mean ± SE DLM weight for the three muscle conditions were: 0.0029 ± 0.0006 g (beige white, $n = 21$); 0.0058 ± 0.0009g (pale pink, $n = 22$); 0.0075 ± 0.0003g (brick red, $n = 67$). Muscles with different colors had significantly different mean weights ($n = 110$, $F_{4,} = 43.283$, $P = 0.0001$). As predicted, functional red muscles were heavier than nonfunctional white muscles.

Phenotypic Correlations.—Both phenotypic correlations were highly significant; call duration and wing morph, $r = -0.61 \pm 0.05$, $n = 218$, df = 1, $P = 0.0001$; LW call and DLM weight, $r = -0.32 \pm 0.09$, $n = 109$, df = 1, $P = 0.001$.

TABLE 3. Summary of genetic correlation estimates ($r_A \pm SE$). All correlations are significantly different from zero.

	Wing morph	DLM weight
LW call duration	−0.46 ± 0.20	−0.80 ± 0.14
SW call duration	−0.68 ± 0.16	

Heritability and Genetic Correlation Estimates

All heritability estimates were significant, except for the offspring-parent heritability of call duration for SW crickets (Table 2). The full-sib and offspring-parent heritability estimates for SW and LW call duration, SW + LW call duration, and DLM weight were very similar, indicating that nonadditive genetic variance (dominance and epistasis) contributes relatively little to the heritability estimates.

Three of the four genetic correlations were highly significant (Table 3). As predicted, with an increase in proportion macroptery in a family the mean SW and LW call duration decreases (Fig. 2). In addition, for LW males in each family, as the mean DLM weight increases the mean LW call duration decreased (Fig. 3). The significant genetic correlation demonstrates a genetic basis to the trade-off. The genetic correlation between wing morphs for call duration was nonsignificant ($r_A = 0.13 \pm 0.22$, $n = 22$, df = 1, $P = 0.561$), suggesting that selection for call duration in one morph will not be constrained by the other morph. However, the large standard error does not preclude an evolutionarily important genetic correlation: a larger sample size is required to answer this question.

DISCUSSION

Although flight capability can be advantageous under conditions of habitat change, it is not without costs. Producing and maintaining the flight apparatus (wings, wing muscles, and flight fuels) is energetically expensive (Mole and Zera 1993, 1994a,b; Tanaka 1993; Zera et al. 1994). The energy needed to maintain the flight muscles is hypothesized to constrain resources that could be used for calling in males. Previous studies on male *G. firmus* have shown the existence of a phenotypic trade-off between macroptery and calling (Crnokrak and Roff 1995) and that the trade-off exists under

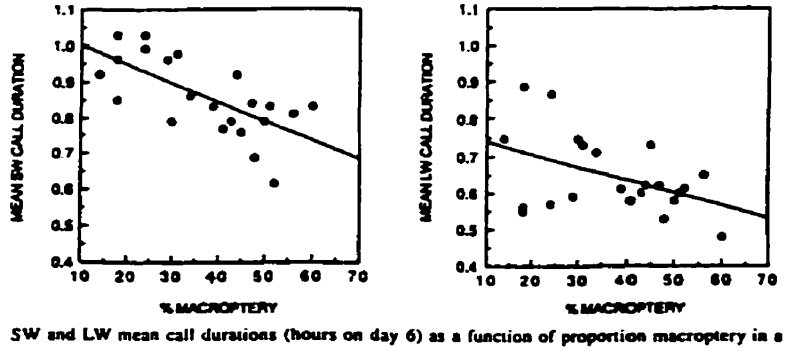

FIG. 2. SW and LW mean call durations (hours on day 6) as a function of proportion macroptery in a family.

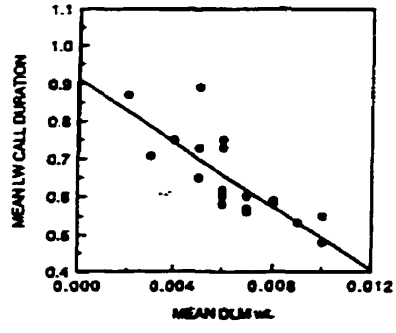

FIG. 3. LW mean call duration as a function of DLM (dorsal longitudinal muscle) weight (g).

both ad libitum food and resource restriction (Crnokrak and Roff, in press). As in the previous study, in the present study we found a significant phenotypic trade-off between macroptery and the probability of attracting a female: SW males attracted a significantly greater number of females than LW males. This difference is due to differences in relative call duration between the morphs: SW males called longer during a 24-h period than LW males. In addition, females preferred the SW male even when call effects were accounted for in the model. On a genetic level, we found that the traits involved in the trade-off (call duration, wing morph, flight muscle weight) are all significantly heritable. The genetic correlations between the traits (LW call duration and wing morph, SW call duration and wing morph, LW call duration and flight muscle weight) were also all significant and all negative. This is, to the best of our knowledge, the first study of trade-offs between a dimorphic trait and fitness-related traits in males to have demonstrated the existence of a genetically based trade-off.

Trade-offs are a central facet of life-history evolution (Stearns 1977, 1989; Bell 1980; Reznick 1985; van Noordwijk and de Jong 1986; Pease and Bull 1988). The study of trade-offs is important because traits rarely evolve as single units. In addition, because there is usually a finite amount of resources available to be allocated to different functions in an organism, competition among traits for resources is inevitable (Pease and Bull 1988). The most commonly measured trade-offs are phenotypic because analyses on this level are relatively simple compared to a genetic analysis (Stearns 1989). A genetic analysis of trade-offs requires heritability estimates of the individual traits involved and genetic correlation estimates between these traits. The present study has fulfilled both of these requirements. Our comparison of full-sib and parent-offspring heritability estimates revealed that nonadditive effects contribute little to the heritability estimates; in addition to the relatively high heritability estimates, this would mean that the traits in question will rapidly respond to selection.

The genetic correlations between call duration and wing morph reported here indicate that the trade-off is relatively high in magnitude and negative. Therefore, in a relatively unstable habitat, although selection will favor macropterous males, because of the negative genetic correlation between wing morph and call duration, selection favoring the increase in proportion macroptery in a population will result in a mean decrease in call duration among the macropterous males. Thus, macropterous males from a population that is predominately micropterous will have relatively longer call durations than macropteorus males from a population that is predominately macropterous. Because longer-calling males have a higher probability of attracting females, variation in proportion macroptery will result in changes in the relative fitnesses between micropterous and macropterous males. In this way, these genetically based trade-offs will mediate the evolution of wing dimorphism in a population.

To fully understand a biological system, it is crucial to examine the phenomenon in question on all possible levels. The behavioral trade-off between macroptery and calling behavior in *G. firmus* males has been studied by us on a phenotypic and genetic level. Previous studies have shown that a behavioral trade-off exists and that this trade-off is affected by available resources (Crnokrak and Roff 1995, in press). Although the present study examines the trade-off on the single most important level, the genetic level, there is still a lack of information concerning how genetics is translated into the phenotype. Because the trade-off is believed to be a resource-based trade-off where a finite amount of resources must be partitioned between maintaining the flight apparatus and calling, it is important to verify this hypothesis by examining the trade-off on a physiological level. A physiological trade-off can be negated on a phenotypic level if an organism has the ability to mediate its behavior to compensate for a shortfall in metabolic resources. In *G. firmus*, macropterous females partially compensate on a behavioral level for the physiological trade-off between macroptery and egg production by eating more food than micropterous females (Mole and Zera 1994a). Although in one experiment with *G. firmus* LW females appeared to compensate for the physiological trade-off by eating more than SW females (Mole and Zera 1994a), this has not been observed in four other experiments with *G. firmus* (Roff 1984, 1989, 1994a; Roff et al. 1997) and one with *G. rubens* (Mole and Zera 1993). We are presently analyzing data from the physiological experiments to determine which traits are involved in the macroptery-call trade-off in male *G. firmus*. To date, we have determined that significant negative genetic correlations exist between call duration and flight muscle weight (this paper) and flight muscle weight and proportion macroptery (Crnokrak and Roff, unpubl. data). As hypothesised, these results confirm that the phenotypic trade-off is underpinned by a trade-off on a physiological level.

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Chapter 6. The influence of lipid stores and flight muscle condition on call duration and its costs in males of the cricket, *Gryllus firmus*.

Having established that a trade-off to macroptery exists on a phenotypic and genetic level and that the trade-off will most likely be important under conditions experienced in the wild, the final chapter in this thesis will attempt to answer the question: how do traits most proximate to the energetics of the trade-off function within the trade-off. Traits that are most proximate to the energetics of the trade-off (lipids and flight muscles) are predicted to exhibit greater trade-offs with calling than traits more distant from the energetics of the trade-off (wing morph). This chapter was written for publication in Oecologia.

Abstract.-Among the Orthoptera, wing dimorphism where one morph is long-winged (macropterous) and flight capable, while the other is short-winged (micropterous) and flight incapable, is relatively common. In addition to long wings, macropterous individuals also produce and maintain a functional flight apparatus, which includes dorsal longitudinal flight muscles (DLM) and flight fuels. Although macropterous individuals can fly and therefore avoid successional changes in their habitat, they are at a energetic disadvantage in terms of fecundity: macropterous females produce fewer eggs than micropterous females. In the cricket, *Gryllus firmus,* in addition to the fecundity trade-offs demonstrated in females, there exist trade-offs to macroptery in males. Previous studies have shown that macropterous males call less and therefore attract fewer females. In this study we examine variation in the primary traits involved in the trade-off; flight muscles and lipid stores. We find that as DLM condition goes from a non-functional to a functional state call duration decreases, and as relative lipid weight increases call duration increases in both morphs. When crickets are subject to a resource stress, mean call duration, mean lipid weight and mean body weight all decreased significantly. We found that the traits examined, body weight and lipid weight, had significant heritabilities. Although our previous studies found significant genetic correlations between call duration and proportion macroptery and call duration and DLM weight, for this study, none of the measured genetic correlations were found to be significant. Traits that are most proximate to the energetics of the trade-off (DLM condition and call duration), show less variability in the trade-off (more negative correlations), compared to traits more distant to the trade-off (wing morph and call duration).

Discrete morphologies exist in a wide range of species (Roff 1996). In many cases dimorphisms in body structure are also associated with behavioural modifications that frequently play an important role in dispersal and in the ability to defend a territory or acquire mates (Crnokrak and Roff 1995). A common dimorphism in insects is wing dimorphism where one morph, macropterous, is long-winged and flight capable while the other morph, micropterous, is short-winged and flightless (Harrison 1980; Roff 1986, 1994a). Wing dimorphisms are believed to be maintained in populations due to trade-offs between the advantages of dispersal and the costs to maintaining the flight apparatus (Roff 1984, 1986; Denno et al. 1991; Roff and Fairbairn 1991). Among wing dimorphic insects, trade-offs between macroptery and reproductive potential have been well studied in females. In general, micropterous females reproduce earlier and have a higher fecundity than macropterous females (Roff 1986; Denno et al. 1989; Roff and Fairbairn 1991). Although there are many examples of phenotypic trade-offs between body morphology and reproductive potential in females, in only two species has it been determined that the trade-offs have a genetic basis (Roff 1990, 1994b; Roff and Bradford 1996; Roff et al. 1997). In males of dimorphic insects, few examples of a trade-off between reproductive fitness and wing morphology have been demonstrated (for a review see Crnokrak and Roff 1995), and only one study has established a genetic basis to the trade-off (Crnokrak and Roff 1998a).

Because many trade-offs probably involve a constraint on the amount of resources that can be partitioned between different functions, examination of traits directly involved in the trade-off requires an investigation of the internal resource store of morphs. The trade-off we are concerned with here is that between wing morph and call duration in males of the cricket *G. firmus*. Macropterous males, due to the energetic costs associated with having to maintain the flight apparatus (wings, flight muscles, flight fuels), are hypothesized to be energetically constrained with respect to the energy needed to produce the energetically expensive calling song (Bailey et al. 1993). Macropterous males are predicted to call less than micropterous males and therefore attract fewer females. Previous studies have shown that a phenotypic trade-off exists (Crnokrak and Roff 1995) and that the traits involved have a genetic

basis (Crnokrak and Roff 1998a). The trade-off is believed to be mediated by energetic resources partitioned between flight muscle maintenance and calling. Large muscles are believed to be more taxing to maintain than small muscles (Mole and Zera 1993, 1994; Zera et al. 1997). In addition to size, functional muscles (indicated by a red colour for presence of mitochondria and cytochrome; Ready and Josephson 1982; Shiga et al. 1991; Gomi et al. 1995) are hypothesized to be much more costly to maintain than partially functional or non-functional muscles (Mole and Zera 1993; for a review see Zera et al. 1997). Energetic resources in crickets include carbohydrates and lipids. Because carbohydrates are used up soon after activity (Nachtigal 1974; Crabtree and Newsholme 1975), it is primarily lipids that make up the energetic resources of individuals (Beenakkers et al. 1981; Rankin and Burchsted 1992). Lipid flight fuels (triglycerides) are hypothesized to be energetically expensive to synthesize because of their high caloric content (Stryer 1988). Previous studies on migratory insects have found higher total lipid and triglyceride content of migratory vs. sedentary adult phases of a number of species (Uvarov 1966; Nwanze et al. 1976; Gunn and Gatehouse 1987). In *G. firmus*, macropterous crickets were found to have 38% higher triglyceride levels than micropterous crickets, while non-triglyceride lipid levels did not differ between morphs (Zera and Mole 1994; Zera et al. 1994). In addition, a negative correlation between triglyceride and non-triglyceride lipid was found in macropterous but not micropterous crickets, suggesting that a trade-off exists due to a constraint in the total amount of lipid that can be biosynthesized (Zera et al. 1994). Therefore, if macropters have high triglyceride levels compared to non-triglycerides, then total lipid content is predicted to be greater in macropterous males than micropterous males. But if triglyceride levels are relatively low, then total lipid content may be only slightly larger or equal in macropterous as compared to micropterous males. Because non-triglyceride lipids are also believed to be the primary energy source for calling, there should also be a positive relationship between relative lipid weight and time spent calling. What is not known is whether these traits are involved, whether they have a genetic basis and whether these traits operate within the trade-off as hypothesized.

We hypothesize that an evolutionarily important trade-off to macroptery exists in male *G. firmus* that involves the primary components of the flight apparatus (wings, flight muscles) and the internal resource stores (lipids). Specifically, we address the following hypotheses:

1) a trade-off exists between flight muscle condition and time spent calling. Our previous study in *G. firmus* males demonstrated a negative genetic correlation between DLM weight and call duration (Crnokrak and Roff 1998a). But large muscles are not always functional, and these non-functional muscles do not require as much resources to maintain compared to functional muscles. Because functional muscles require more resources to maintain in a working state, fewer resources will be available for calling, therefore, crickets with functional muscles should call less.

2) Because lipids are the primary energy stores in crickets and the primary energy used for calling, then crickets with larger lipid stores are predicted to call longer than crickets with smaller lipid stores. Due to the complications of triglyceride and non-triglyceride lipid levels, we make no *a-priori* prediction as to which wing morph will have a higher total lipid content.

3) Crickets subject to a resource stress are hypothesized to have more pronounced trade-offs, because restricted resources are believed to exacerbate the resource limited condition in macopterous males. Resource stressed crickets should have lower lipid stores and body weights than *ad libitum* fed crickets.

4) The variability of the trade-off is predicted to decrease as one examines traits that are most proximate to the basis of the trade-off. Specifically, we predict that the negative correlation will be greater between call duration and DLM condition than between call duration and wing morph because the correlation is predicted to decline with distance from the trait due to the addition of environmental variance.

5) All of the traits involved in the trade-off: body weight and lipid weight, are predicted to have a genetic basis and be genetically correlated with DLM weight and call duration. For this paper we have used the data of flight muscle weight, flight muscle condition, body weight and lipid weight obtained from crickets used for the previous three studies dealing with

trade-offs to macroptery in *G. firmus* males (Crnokrak and Roff 1995, 1998a,b).

Materials and methods

1. Species description and methods of rearing

For simplicity we shall designate the three experiments as Expt 1 (Crnokrak and Roff 1995), Expt 2 (Crnokrak and Roff 1998a) and Expt 3 (Crnokrak and Roff 1998b). For general rearing conditions for the individual experiments of crickets see Crnokrak and Roff (1995). On the day of their final ecdysis (the day nymphs became adults), we placed males in individual buckets containing, 1) *ad libitum* food and water (Expts 1 and 2), or 2) restricted amount of food (0.03 g every third day) and *ad libitum* water (Expt 3).

2. Experimental design

2.1. Call monitoring for Expts 1, 2 and 3.- Following final eclosion, we placed each male in an individual glass jar (9cm in diameter) with *ad libitum* food and water (restricted food for Expt 3). Crickets monitored for Expts 1 and 2 were monitored for one day, day six of adult life. We used crickets that were six days old because preliminary analysis indicated that by this day call duration levels off and remains constant thereafter (Cade and Wyatt 1984; Crnokrak and Roff 1998b). Crickets monitored in Expt 3 were measured every day from the first day of eclosion up to 20 days as adults. We monitored crickets for 20 days only since field studies on rangeland grasshoppers have shown that mortality is about 5% per day (Onsager and Hewitt 1982); thus after 20 days, most individuals in a population are dead. We used a continuous playback of cricket calling to provide a constant background in each incubator because male crickets tend to call more often when they hear others call (Cade and Wyatt 1984).

We monitored male calling using a Realistic tie-clip 33-105 microphone (frequency response, 50-15000 Hz) placed in each male's jar. Microphones were connected to an analog-to-digital converter relay system monitored by a computer that recorded the time of each incoming signal. The gain of each microphone was set at a level that would trigger the relay system only when the occupant of the jar called and not by the

background call or the calls of neighbouring crickets. For one day of monitoring, each male was monitored for a 23.5h.

We used 47 males (22 LW, 25 SW) in Expt 1. Three SW males were monitored for call duration, but since they were not paired up with LW males, were not scored for female attraction (for details see Crnokrak and Roff 1995). For Expt 2, we set up 22 families with 5 SW and 5 LW males measured per family. A total of 110 SW and 110 LW offspring were measured (for details see Crnokrak and Roff 1998a). For Expt 3, we used 6 SW and 6 LW crickets for the control group and 15 SW and 15 LW crickets for the food restricted group (for details see Crnokrak and Roff 1998b).

2.2. DLM dissection and lipid extraction.-All crickets were preserved by freezing. Once thawed, crickets were dissected to remove the dorsal longitudinal flight muscles (DLM) that are immediately below the dorsal mesothoracic covering (Du Porte 1920; Srihari et al. 1975; Pfau and Koch 1994). Although all crickets were dissected, only LW crickets had measurable DLM, therefore, all SW DLM weights were scored as 0. We assessed DLM condition on a three point scale: 0-white, 1-pink, and 2-brick red. A red colour indicates the presence of mitochondria which means the muscles are functional (Mole and Zera 1993; Zera et al. 1997). All colour tests were done by one person (P.C.) to avoid any discrepancies between experimenters. Once the DLM were dissected, they were placed on a numbered, pre-weighed, microscope coverslip placed in an oven set at 60 ^{0}C for at least 3 days. Once dried, DLM were measured on a Mettler digital scale to 4 decimal places. After the DLM were dissected out, the crickets were placed in 25ml pre-weighted vials with a 10ml solution of 2:1 vol:vol methanol:chloroform (Lee et al. 1975). Crickets were left in this solution for at least seven days to ensure that all of the lipids precipitated out into the solution. The solution precipitates out all of the different lipids found in crickets (triglycerides, phosolipids, sterols and hydrocarbons) (Lee et al. 1975). Before being removed from the vials, crickets were washed with approximately 1ml of solution to ensure that no lipids adhered to the bodies. The crickets were then placed on pre-weighed coverslips in an oven set at 60 ^{0}C for approximately one week. After one week the cricket's dry body weight was measured on a Mettler scale to 4 decimal

places. The remaining vials with lipids and solution were placed in the same oven to evaporate off the chloroform/methanol solution leaving the lipids in the vial. Lipid weight was measured as the difference in weight between vial weight + lipid weight subtracted from the previously measured vial weight.

3. Statistical analyses

3.1. Hypotheses 1 and 2.- We grouped Expts 1 and 2 together since all variables were measured on day 6 and analysis indicated no difference between these two experiments. We assessed the effect of body weight, DLM condition, lipid weight and experiment on call duration for the combined Expts 1 and 2 using the multiple regression model (coefficients omitted):

call duration=constant + body wt. + DLM condition + lipid wt. + experiment + interactions (1),

where *interactions*=all 2-way interactions of the independent variables with *experiment (body wt. x experiment, DLM condition x experiment, lipid wt. x experiment).* DLM condition is a categorical variable. We excluded Expt 3 since crickets measured for this experiment were measured on day 20. We ran model 1 for LW and SW males separately because SW males were not scored for DLM condition. The remaining interactions between the independent variables *(body wt. x DLM condition, body wt. x lipid wt.* and *DLM condition x lipid wt.)* were tested using Spearman correlations (SW and LW males grouped together) to test for colinearity. Testing for colinearity among the independent traits is important when the relationship between one trait is measured against many independent traits that are strongly correlated with each other.

We also calculated the Spearman correlation between DLM weight and DLM condition using Expts 1 and 2 to determine if functional muscles are larger than non-functional muscles.

3.2. Hypothesis 3. -To determine if a resource stress affects lipid weight we analyzed the effect of body weight, wing morph and treatment on lipid weight using the regression model for Expt 3:

lipid weight=constant + body wt. + morph + treatment + interactions (2).

We also analyzed the effect of wing morph and treatment on body weight using Expt 3 and the regression model:

body wt.=constant + morph + treatment + interactions (3).

The analysis of models 2 and 3 included both LW and SW males.

3.3. Hypothesis 4.- To determine if the magnitude of the trade-off increases with the proximity of the trait to the energetics of the trade-off, we compared phenotypic correlations between 1) call duration and wing morph and between 2) call duration and DLM condition. Correlations were calculated using offspring values from Expt 2. Because family sizes were equal, phenotypic correlations were calculated using a general linear regression. We compared the phenotypic correlations between call duration and DLM condition calculated here with the previously published phenotypic correlation between call duration and wing morph (Crnokrak and Roff 1998a) using Z-scores (Sokal and Rohlf 1981). Because we predict, *a-priori,* that the correlation between DLM condition and call duration will be greater than the correlation between wing morph and call duration, the test was 1-tailed.

3.4. Hypothesis 5.- Full-sib heritability estimates were calculated using nested (cages nested within families) ANOVA and the Jackknife (Simons and Roff 1996). As both estimates gave almost identical results we present only those from the ANOVA. Heritability estimates calculated were: dry body weight and lipid weight. Cage effects were non-significant and, therefore, cages were combined.

We calculated genetic correlations for: 1) lipid weight and SW+LW call duration, 2) lipid weight and DLM weight, 3) lipid weight and wing morph, and 4) DLM weight and wing morph. Because a fixed number of LW and SW offspring per family, irrespective of proportion macroptery in the family, were measured, genetic correlations could not be estimated in the usual manner (Roff and Bradford 1996). However, genetic correlations from full-sib data can be estimated, at least approximately, by the Pearson product moment correlation between family means (Via 1984; Roff and Preziosi 1994). Mean values per family were calculated by first calculating mean values per cage (as described above) and then averaging across cages.

Standard errors values for the correlation estimates were calculated using (Becker 1995):

$$S.E.=\sqrt{\frac{1-r^2}{n-2}}.$$

Results

1. Hypotheses 1 and 2: Crickets with non-functional muscles and large lipid stores should call longer than crickets with functional muscles and small lipid stores.

The results of the regression analysis using Expts 1 and 2 revealed significant effects of all of the independent variables for both the SW and LW male analyses (Table 1). The two way interactions with *experiment* were all significant, except for *body wt. x experiment* in SW males and *DLM condition x experiment* in LW males, indicating that there are significant differences between Expts 1 and 2 in the relationship of the independent variables with call duration. For LW males, as predicted, call duration decreases as DLM condition varies from non-functional to functional (Table 1, Fig 1). In addition, for both SW and LW males, as was hypothesized, call duration increases as lipid weight increases (Table 1, Fig 2). The significant relationship between call duration and body weight for Expts 1 and 2 changed direction in SW and LW males: in SW males the relationship is positive, in LW males it's negative (Table 1). None of the correlations between the independent variables (*body wt.* and *DLM condition, body wt.* and *lipid wt., DLM condition* and *lipid wt.*) were significant, indicating no significant effects of colinearity between the traits. As predicted, functional muscles were significantly heavier than non-functional muscles (r=0.57±0.07, n=131; Fig 1c).

2. Hypothesis 3: Lipid and body weights should be lower in ration-limited crickets.

As predicted, mean lipid weights were significantly greater in the control group than the food restricted group and SW males had significantly larger lipid weights than LW males (control=0.050±0.012, 0.022±0.012 (SW, LW); resource restricted=0.001±0.001, 0.001±0.0001 (SW, LW); Table 2). *Wing morph* and *treatment* had highly significant effects on

body wt. (see Table 2 for details). SW males had significantly larger body weights than LW males (Table 2). As predicted, the food restricted group had significantly lower body weights than the control group for both wing morphs (control=0.200±0.012g, 0.153±0.020g (SW, LW); resource restricted=0.129±0.006, 0.113±0.005 (SW, LW)).

3. Hypothesis 4: Correlation between call duration and DLM condition will be greater than between call duration and wing morph.

As predicted, the correlation between call duration and DLM condition (r=-0.86±0.03, n=131) was significantly larger (Z=5.24, P=0.0001) than the previously reported correlation between call duration and wing morph (r=-0.61±0.05, n=219).

4. Hypothesis 5: Traits will be heritable and genetically correlated.

All traits hypothesized to be involved in the trade-off have significant heritabilities. The heritability estimates for both body weight (0.26±0.13, n=219, P=0.05) and lipid weight (0.17±0.07, n=219, P=0.04) were both significant (for heritabilities of wing morph, call duration and DLM weight see Crnokrak and Roff 1998a).

None of the genetic correlations measured for this study were significantly different than zero (see Crnokrak and Roff 1998a for other significant correlations). Since the non-significant correlations are all relatively small (call duration and lipid weight=0.09±0.22, lipid weight and DLM weight=0.14±0.22, lipid weight and wing morph=0.04±0.22, DLM weight and wing morph=0.19±0.22), much larger sample sizes are needed to obtain reasonably low standard error values.

Discussion

Previous studies on *G. firmus* males have demonstrated that macropterous males call less and attract fewer females than micropterous males (Crnokrak and Roff 1995). In addition, the traits involved in this trade-off, wing morph and call duration, had significant heritabilities and significant genetic correlations (Table 2 and 3, Crnokrak and Roff 1998a). For this study, we have demonstrated that an evolutionarily important trade-off exists that involves the energetic resources of male *G. firmus* (lipids stores). The data for this study originated from the dissection of crickets used from previous experiments on trade-offs to macroptery (Crnokrak and Roff 1995; 1998a,b). As

hypothesized, trade-offs exist between DLM condition and call duration for the combined Expts 1 and 2: crickets with functional DLM, had significantly lower mean call durations than crickets with non-functional DLM (Fig 1a&b). Lipid weight had significant effects on call duration for both experiments; as hypothesized, call duration increases as lipid weight increases (Fig 2a&b). For the resource restricted crickets, significant effects of wing morph and treatment on lipid weight were found: the control group had significantly larger lipid weights than the food restricted group for both SW and LW males. Body weight was also found to be significantly different between morphs for the resource restriction experiment with SW males having larger body weights than LW males (Table 2), indicating that LW males maybe more energetically stressed than SW males. In addition, food restricted crickets had lower body weights than *ad libitum* fed crickets. We also found that the variability in the trade-off decreased as we examined traits that were more proximate to the energetics of the trade-off; the correlation between call duration and DLM condition was significantly greater than the correlation between call duration and wing morph. The heritability estimates for body weight and lipid weight were significantly different than zero. Although our previous study found significant genetic correlations between wing morph and call duration and call duration and DLM weight (Crnokrak and Roff 1998a), none of the genetic correlations measured for this study were significantly different than zero. All of the genetic correlations had relatively high standard errors. Although previous studies have examined the energetic costs to macroptery in male *G. firmus* (Zera et al. 1994, 1997), this study is the first to demonstrate directly that an energetic trade-off exists to macroptery in males of a dimorphic insect. In addition, to the best of our knowledge, these are the only quantitative data available in a male dimorphic insect to demonstrate the fitness gain due to microptery in comparing flight muscle variation and lipid weight.

We found significant variation in DLM weight and DLM condition between morphs and between experiments. Muscles develop during the last instar of nymphal development (Roff 1989). Once fully developed DLM can vary dramatically among females of the same age (Young 1965; Pener 1985; Roff 1989). When crickets are six days old, 50% of

the LW *G. firmus* females show signs of wing muscle histolysis (Roff 1989; Zera et al. 1997). By day five, all female SW crickets have histolyzed muscles (Zera et al. 1997). Similar patterns of developmental changes in DLM were found in males (Zera et al. 1997). Once DLM histolyze, the trade-off is believed to be substantially reduced because the major resource constraint to fecundity is now no longer an important consideration (Zera et al. 1997). Our experiments have shown that in male *G. firmus*, DLM weight when measured on day 6 of adult life is substantially larger (0.006g compared to 0.001g) than when measured on day 20 (Fig. 1c). Since we assessed DLM weight on only 2 different days (day 6 and 20), we were not able to determine when muscles start to be histolyzed. Nevertheless, when using DLM condition to assess degree of histolysis, we found that by day 6, 41% of LW males dissected showed signs of histolysis. We also found that a significant relationship exists between LW call duration and DLM condition for the combined Expts 1 and 2. As DLM condition varied from non-functional to functional, call duration for LW males decreased (Fig 1a&b). We cannot explain why the slopes of the relationship between LW call duration and DLM condition are different for the phenotypic and genetic experiments (Expts 1 and 2 respectively). Due to DLM histolysis, the relationship between LW call duration and DLM weight for the resource restriction experiment is non-significant because by day 20 of adult life the wing muscles have been fully histolyzed. The phenotypic correlation between DLM weight and LW call duration previously published was -0.32±0.09 (Crnokrak and Roff 1998a). We found that the trade-off with call duration was much less variable when we used DLM condition (r=-0.86±0.03) in place of DLM weight or wing moph (r=-0.61±0.05). In addition, we found that functional muscles are large while non-functional muscles are small (r=0.57±0.07). This is a similar finding to other published studies of muscle condition and weight in *G. firmus* (Zera et al. 1997). To the best of our knowledge this is the first study to have published the relationship between DLM condition and call duration in a wing dimorphic insect.

Although the relationship between call duration and DLM condition seems relatively clear, the same is not necessarily true for call duration and lipid weight. Although lipids are believed to be the primary energy resources used for calling, interpretation of our experiments is

complicated by the fact that there are different types of lipids used for different functions. Crickets use non-triglyceride lipids for use in the daily energy budget, while macropters are known to synthesize triglycerides specifically for flying (see introduction). Since triglycerides are believed to be energetically expensive to produce, and because they are part of the flight apparatus, they are therefore important to the trade-off. The method we have used to extract lipids from crickets does not allow us to separate non-triglyceride lipids from triglycerides. This complicates matters since LW males may have larger total lipid stores than SW males, but not all of the lipids may be used for calling. Since Zera et al. (1997) demonstrated a negative correlation between triglyceride and non-triglyceride levels in macropterous *G. firmus*, micropterous males may have greater lipid stores for calling. On the other hand, under resource restricted conditions, LW males may be at an energetic advantage compared to SW males since they may be able to use the triglyceride lipids to satisfy their daily metabolic needs. Despite these complications, we found significant relationships between call duration and lipid weight for Expts 1 and 2. In addition, the relationship was in the predicted direction for these experiments. As relative lipid weight increased, so did call duration. An interesting finding was that no significant difference within morphs in mean lipid weight was found between the three experiments. Expt 1 and 2 crickets were measured on day six while the resource restricted crickets were measured on day 20. This suggests that lipid weight was invariant with age for SW males. For LW males there was a decrease with age but it was not significant. The resource restricted crickets, on the other hand, had substantially lower lipid weights, as was hypothesized. Although lipid weight has a significant heritability, we found no significant genetic correlations with call duration, DLM weight and wing morph. This does not mean that no such correlations exist, because the non-significant result could be due to low sample sizes.

One important observation that becomes apparent from our studies is that the relative variability of the trade-off to macroptery changes depending on which traits are examined. The correlation between call duration and wing morph previously measured was -0.61±0.05 (Crnokrak and Roff 1998a), indicating that a moderately high

phenotypic correlation exists between morphological traits that are relatively far removed from the energetic trade-off to macroptery. When we measured traits that were directly associated with the trade-off, we find that the correlation is much greater in magnitude (call duration and DLM condition=-0.86±0.03). Thus our previous estimate of the variability of the trade-off to macroptery in male *G. firmus* was an underestimate. Similar underestimates were found in *G. firmus* females where fecundity was much higher (50% greater gain) in females with white versus red DLM than was in females that were micropterous versus macropterous (Zera et al. 1997). Although we do not know whether DLM condition is heritable (we cannot calculate a heritability for this categorical variable), the correlation comparisons using DLM condition versus wing morph indicated that DLM condition was the more informative of the two traits. This makes sense since we found that large muscles were sometimes categorized as pink or white which indicates that they are partially functional or non-functional. The metabolic and energetic needs of these large, non-functional muscles is going to be much lower than large functional muscles. Therefore using call duration and DLM condition allows us to estimate the magnitude of the macroptery trade-off in male *G. firmus* more accurately than using DLM weight, and much more accurately than using wing morph.

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Table 1 Regression analysis to test the effect of body weight, DLM condition, lipid weight and experiment on call duration using the model: *call duration=constant+body wt.+DLM condition+lipid wt.+interactions.* Separate analyses are for SW and LW males of Expts 1 and 2 (n=131). Note: *body wt.*=dry body weight (g); *expt*=0(Expt 1), 1(Expt 2)

variable	*coefficient*	*t*	*P*
SW males			
body wt.	11.893	2.072	0.040
lipid wt.	16.687	5.487	0.00001
expt.	1.819	2.558	0.012
body wt. x expt.	-11.363	-1.915	0.058
lipid wt. x expt.	-16.469	-5.378	0.00001
LW males			
body wt.	-16.346	-5.000	0.0001
DLM condition	-0.064	-1.543	0.050
lipid wt.	16.422	8.579	0.00001
expt.	-1.278	-2.917	0.0042
body wt. x expt.	15.807	4.638	0.00001
DLM condition x expt.	-0.017	-0.367	0.714
lipid wt. x expt.	-16.401	-8.475	0.00001

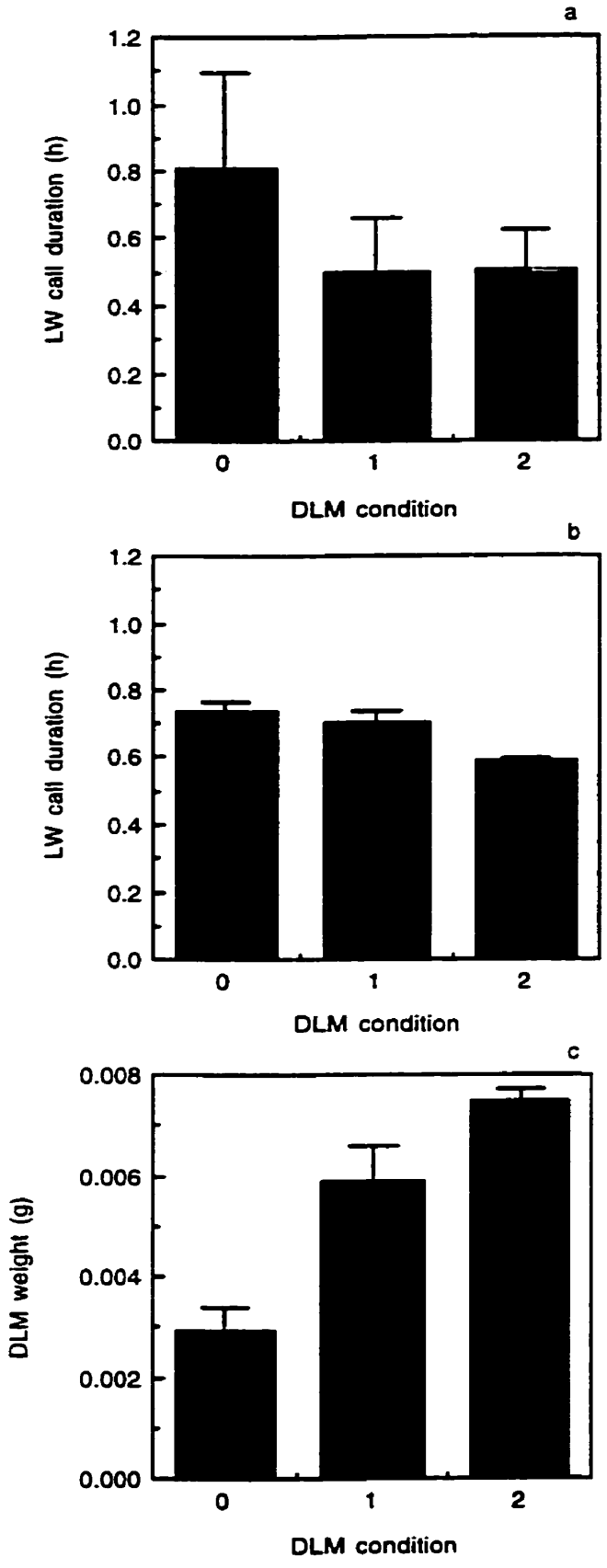
Table 2 Analysis of Expt 3 to test the hypothesis that restricted resources increase the magnitude of the trade-off. Note: *morph*=0 (LW), 1 (SW); *treatment*=0 (control), 1 (resource restricted)

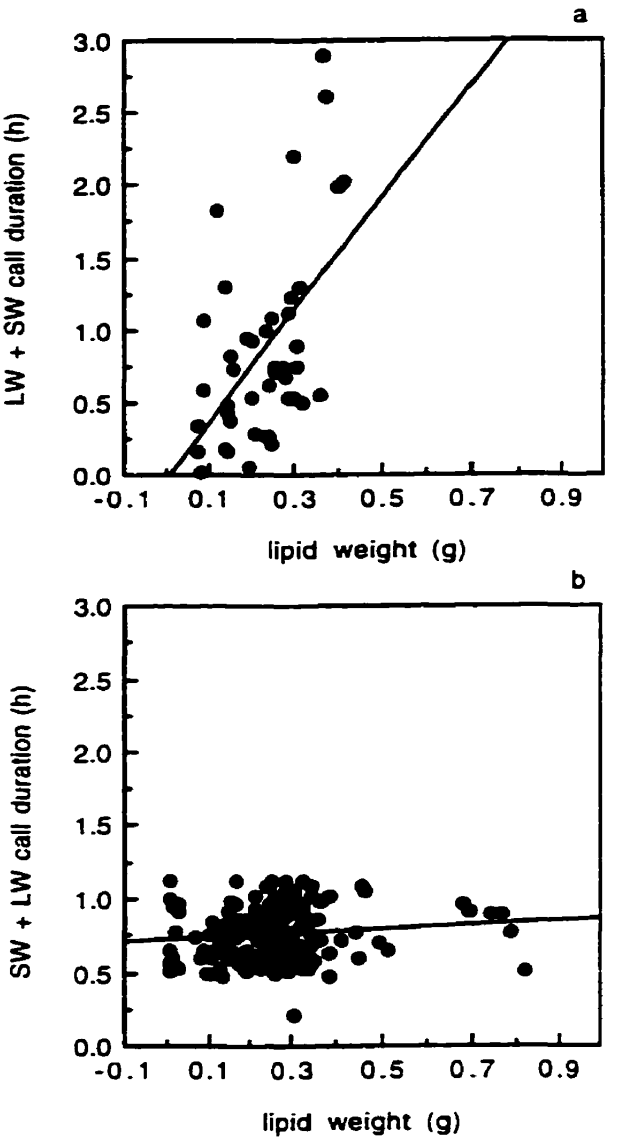
variable	*coefficient*	*t*	*P*
model 1: *lipid weight=constant+body wt.+morph+treatment+interactions*			
morph	0.171	2.896	0.004
body wt.	0.316	2.002	0.055
treatment	-0.015	-1.968	0.030
morph x body wt. x treatment	0.696	1.740	0.091
morph x body wt.	-0.992	-2.505	0.017
morph x treatment	-0.137	-2.311	0.027
model 2: *body weight=constant+morph+treatment*			
morph	0.017	2.629	0.012
treatment	-0.021	-2.908	0.006

Figure Captions

Fig. 1 LW call duration (in hours) as a function of DLM condition for a) Expt 1, b) Expt 2, and c) DLM weight as a function of DLM condition. Note: DLM condition: 0=non-functional, 1=partially functional, 2=fully functional.

Fig. 2 Call duration (SW and LW in hours) as a function of lipid weight for a) Expt 1, b) Expt 2.


a
LW call duration (h)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
1
2
DLM condition
b
LW call duration (h)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
1
2
DLM condition
c
DLM weight (g)
0.008
0.006
0.004
0.002
0.000
0
1
2
DLM condition


a
LW + SW call duration (h)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.1
0.1
0.3
0.5
0.7
0.9
lipid weight (g)
b
SW + LW call duration (h)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.1
0.1
0.3
0.5
0.7
0.9
lipid weight (g)

General Conclusion

Studies of life history evolution have shown that trade-offs are one of the primary factors involved in the evolution of traits closely related to fitness (Stearns 1977, 1989; Reznick 1985; Peace and Bull 1988). Despite the fact that phenotypic trade-offs have been well documented (see Stearns 1989 for examples), some studies have shown no significant trade-offs, while a number of studies have shown positive correlations between fitness related traits (Garland 1988; Brodie 1989, 1991, 1993). Experiments that involve the artificial manipulation of traits are more successful at measuring trade-offs than experiments in which the association of individual traits are simply recorded under laboratory conditions (Bell and Koufopanou 1986; van Noordwijk and de Jong 1986; Tatar and Carey 1995). This is presumably because without the artificial manipulation of resources/traits, the benign conditions of a laboratory mask any trade-offs that would normally be present in the wild. For trade-offs to affect the independent evolution of the component traits, they must, (1) be genetically based (heritable component in the independent traits and a negative genetic correlation between the traits) and (2) be prevalent under conditions found in the wild (Stearns 1989). The most commonly measured traits in studies of trade-offs are those most proximate to fitness, for example, reproductive potential or timing events such as age at maturity (Stearns 1989; van Noordwijk and de Jong 1986). Few studies have examined life history trade-offs that involve behavioural traits. For example, a trade-off between territorial defense and survivorship was observed in the lizard *Sceloporus jarrovi* when subjects were injected with testosterone (Marler et al. 1995). In two spider species, *Nephila claviceps* and *N. maculata*, a negative correlation between weight gain and the size of the web spun was found only at high food levels (Higgins 1995). Although examples of behavioural trade-offs exist, very few studies have examined the genetic basis of these trade-offs. This thesis examined the trade-off between wing morphology and call behaviour in males of the wing dimorphic cricket, *Gryllus firmus*. It is the first study to have examined and quantified the genetic basis of the behavioural traits involved in the trade-off between wing morph and the likelihood of attracting a female in males of a dimorphic insect. I also examined how variation in resources (as

would be experienced in the wild) affected the magnitude of the trade-off. This study was also the first in a male insect to demonstrate the fitness gain due to micropteryy in comparing traits most proximate to the energetics of the trade-off (flight muscle variation and lipid weight). Two of the six chapters of this thesis were devoted to examining issues integral, but secondary, to the behavioural trade-off in *G. firmus* males; (1) the effect of selection on the genetic architecture of traits (most importantly dominance variance) and (2) whether inbreeding depression is an important factor in the wild and whether it is greater in the wild than in captive conditions. As hypothesized, traits subject to strong selection had significantly larger dominance variance components than traits subject to weaker selection and inbreeding depression was substantially higher in wild populations than in captive ones.

Although I have established a genetic basis to the component traits in the trade-off and also significant genetic correlations, it is not known if these heritabilities and genetic correlations will be significant in the wild. Although I have demonstrated that under restricted resources in the laboratory, the trade-off is maintained, it is not known if environmental variance would result in non-significant heritabilities and correlations in the wild. Environmental variance in the wild is predicted to increase the phenotypic variance (V_P) component of heritability estimates (Falconer 1989). Because heritability is genetic variance expressed as a function of phenotypic variance, increasing V_P would decrease h^2 estimates. If V_P is large enough, the h^2 estimates will be non-significant. In addition, genetic correlations are believed to be even more sensitive to environmental variance, making them extremely difficult to estimate in the wild (Falconer 1989). Because of this, it is commonly believed that estimates of heritability and genetic correlations in the controlled laboratory setting are not applicable to the situation encountered in the wild. If phenotypic variance is large and heritabilities and genetic correlations are non-significant in the wild, then the component traits will not respond to selection (Falconer 1989). In addition, even if heritability estimates are high, if the total genetic variance is primarily non-additive genetic variance, then the traits will not respond to selection (Fisher 1930; Lynch 1994). A comparison of heritability estimates made in the laboratory and the wild by Weigensberg and Roff (1996) revealed that

not only were wild estimates significantly different than zero, but they were also, on average, larger than laboratory estimates. In addition, the comparison of my full-sib and parent-offspring heritability estimates for call duration revealed that most of the genetic variance is additive (chapter 5). Therefore, the heritability estimates calculated for this thesis, will probably be evolutionarily important in the wild. Whether the same can be said of the genetic correlation estimates, at this time is unknown.

In conclusion, I have successfully demonstrated that a trade-off important to the evolution of wing dimorphism exists in male *G. firmus*. Although I have shown that a trade-off exists between macroptery and the likelihood of attracting a female, and that the component traits involved have a genetic basis, it is still poorly understood how the trade-off is mediated on a physiological level. Wing morph, although a discretely varying trait, is believed to be controlled in part by a continuously varying amount of juvenile hormone (JH; see Roff and Fairbairn 1991 and references therein). JH is not only believed to be a controlling factor in wing morph, but also a number of other traits of the flight apparatus. Flight propensity, degree of development and timing of histolysis of wing muscles and flight fuel synthesis are all believed to be controlled by the titre of JH in a cricket (see Roff and Fairbairn for a discussion). Therefore, selecting on one of these traits (eg. proportion macroptery in a population) will result in a change in the mean JH levels in a population and ultimately affect all of the other traits controlled by JH. Although evidence of such correlated responses to selection exists in female *G. firmus* (see Introduction), no research has been done in males. In addition, it is not known if variation in JH titre affects calling behaviour in males. Research into how JH titre responds to selection is one of the most important aspects involved in the evolution of wing dimorphisms in *G. firmus* because JH has such wide sweeping effects on so many components of the flight apparatus.

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Appendix 1. List of species, trait descriptions, estimates of the component of dominance variance ($D_\alpha = \frac{V_d}{V_d + V_a}, D_\beta = \frac{V_d}{V_p}$) and sources. L=life history, B=behaviour, P=physiology, and M=morphology.

Species	Trait Description	Estimate D_β	Estimate D_α	L	B	P	M	Reference
Out-bred Species								
Apis mellifera (bee)	proboscis extension behaviour		0.00		•			Brandes 1991
Drosophila melanogaster (fly)	live weight	0.00	0.00				•	Clark 1990
	triacylglycerol content	0.00	0.00			•		
	glycogen content	0.00	0.00			•		
	fatty acid synthetase	0.00	0.00			•		
	glycogen phosphorylase	0.00	0.00			•		
	glycogen synthase	0.00	0.00			•		
	glucose-6-phosphate dehyd.	0.47	0.54			•		
	hexokinase	0.49	0.66			•		
	malic enzyme	0.51	0.56			•		
	6-phosphogluconate dehyd.	0.00	0.00			•		
	phosphogluconate isomerase	0.00	0.00			•		
	phosphoglucomutase	0.47	0.52			•		
	trehalase	0.00	0.00			•		
	sternopleural chaetae number		0.02				•	Hill 1964
	viability		0.03, 0.05	•				Suh and Mukai 1991
Gerbera hybrida	length of scape	0.11	0.17				•	Harding et al. 1991
	diameter of disk flowers	0.00	0.00				•	
	diameter to trans florets	0.10	0.20				•	
	diameter of entire inflor.	0.06	0.06				•	
	length of outer corolla of ray	0.18	0.21				•	
	width of outer corolla of ray	0.17	0.25				•	
	length of inner corolla of ray	0.06	0.15				•	
	length of outer corolla of trans	0.19	0.30				•	
	width of outer corolla of trans	0.27	0.34				•	
	length of inner corolla of trans	0.17	0.28				•	
	dry weight of scape	0.07	0.10				•	
	dry weight of inflorescence	0.02	0.03				•	
	flowering time	0.08	0.12	•				
	cut-flower yield	0.01	0.03				•	
	number of leaves	0.26	0.34				•	
	length of leaf lamina	0.21	0.34				•	
	width of leaf lamina	0.35	0.35				•	
	number of growing points	0.01	0.03				•	
Hyla crucifer (tree frog)	initial size	0.70	0.71				•	Travis et al. 1987
	growth rate	0.63	0.81	•				
	length of larval period	0.47	0.84	•				

	size at metamorphosis	0.02	0.03				•	
Lolium perenne (grass)	tolerance to sulfur dioxide		0.85			•		Wilson and Bell 1990
Macropodus operculans (fish)	rapid to-and-fro movement		0.17		•			Gerlai et al. 1990
	fast locomotion		0.11		•			
	slow locomotion		0.05		•			
	staccato motion		0.56		•			
	creeping propulsion		0.13		•			
	air gulping frequency		0.05		•			
	floating		0.34		•			
	oblique plane position		0.43		•			
	motionlessness		0.12		•			
Muscidifurax raptor (wasp)	sex ratio (5 days)	0.01	0.16	•				Antolin 1992
	sex ratio (10 days)	0.04	1.00	•				
	lifetime fecundity	0.18	0.92	•				
	fecundity (5 days)	0.05	0.28	•				
	fecundity (10 days)	0.13	0.84	•				
	reproductive life span	0.05	1.00	•				
	development time	0.07	0.50	•				
Muscidifurax raptorellus (wasp)	% greganous oviposition	0.34	0.45	•				Legner 1991
	eggs per greganous ovipos.	0.59	0.61	•				
	eggs per parasitized host	0.56		•				
Mus musculus (mouse)	cerebellar foliation		0.54			•		Cooper et al. 1991
	locomotor activity	0.02	0.65		•			Henderson 1981
Nicotiana rustica	final height		0.09				•	Jinks et al. 1968
	flowering time		0.12	•				
Papaver somniferum (poppy)	days to flower	0.54	0.63	•				Shukla and Khanna 1992
	days to maturity	0.92	0.70	•				
Picea manana (spruce)	5-year height	0.00	0.01				•	Mullin et al. 1992
	4-year height	0.00	0.00				•	
	5th-year leader length	0.00	0.01				•	
	survival	1.00	1.00	•				
Rattus norvegicus (rat)	escape-avoidance conditioning	0.06	0.23		•			Hewitt and Fulker 1983
Sinapis alba	oleic acid level	0.14	0.15			•		Ecker and Yaniv 1993
	linolec acid level	0.23	0.24			•		
	eicosenoic acid level	0.96	0.68			•		
	erucic acid level	0.07	0.07			•		
Taeniopygia guttata (zebra finch)	bill colour	0.00	0.00				•	Price and Burley 1993
Tribolium castaneum (beetle)	development rate	0.21	0.58	•				Dawson 1965
Tribolium confusum (beetle)	development rate	0.36	0.77	•				
Domestic Species								
Arachis hypogaea (peanut)	plot yield		0.09	•				Halward and Wynne 1991
	20-pod length		0.62				•	
	20-pod width		0.52				•	
	20-pod weight		0.39				•	

	# seeds/20 pods		0.00			•	
	seed weight		0.16			•	
	seed:pod ratio		0.36	•			
Avena sativa (oats)	green fodder yield		0.64			•	Kishor et al. 1992
	dry matter yield		0.60			•	
	crude protein		0.78		•		
	digestibility		0.53		•		
Brassica napus (oilseed rape)	seed:husk ratio		0.35			•	Gupta and Labana 1992
	seed:husk nitrogen ratio		0.00		•		
	nitrogen harvest index		0.00		•		
	harvest index		0.40	•			
	duration of reproduction phase		0.38	•			
Bread wheat	grain yield/plant		0.46	•			Nanda 1990
	tillers/plant		0.00			•	
	grains/spike		0.17			•	
	100-grain weight		0.16			•	
	harvest index %		0.17				
	spike length		0.00			•	
	spikelets/spike		0.63			•	
	plant height		0.15			•	
Cucumis melo (melon)	vine length		0.63			•	Kitroongruang et al. 1992
	days to first harvest		0.73	•			
	# fruits/plant		1.00	•			
	fruit weight		1.00			•	
	fruit weight/plant		1.00			•	
	soluble solids		1.00		•		
	rind firmness		0.23			•	
	flesh		0.14			•	
	shape index		0.26			•	
	net		1.00			•	
	vein tract		1.00			•	
Fragaria x ananassa (berry)	resistance to crown rot	0.60	0.54		•		Creighton and Smith. 1991
	soluble solids content	0.07	0.24		•		Shaw 1990
	titratable acid content	0.17	0.36		•		
Gossypium arboreum (cotton)	days to flower		0.82	•			Tomar and Singh 1992
	plant height		0.42			•	
	bolls/plant		0.00			•	
	seed-cotton yield/plant		0.00			•	
	seed yield/plant		0.00	•			
	lint yield/plant		0.00			•	
	halo length		0.62			•	
	ginning percentage		0.01			•	
	seed index		0.00			•	
	lint index		0.00			•	
Gossypium hirsutum (cotton)	2.5% span length		0.72			•	Nadarajan and Rangasamy 1992

	uniformity ratio		0.75			•	
	fibre fineness		0.78			•	
	maturity coefficient		0.75	•			
Helianthus annuus (sunflower)	plant height	0.10	0.14			•	Miller and Hammond 1991
	stem diameter	0.28	0.59			•	
Holstein Cow	days open	0.02	0.54	•			Hoeschele 1991
	days open (150)	0.01	0.19	•			
	service period	0.01	0.62	•			
	service period (91)	0.03	0.79	•			
	milk yield	0.06	0.13			•	Tempelman and Burnside 1990a
	fat yield	0.24	0.43		•		
	final score	0.15	0.50			•	Tempelman and Burnside 1990b
	general appearance	0.12	0.47			•	
	dairy character	0.03	0.11		•		
	capacity	0.16	0.36			•	
	rump	0.07	0.23			•	
	feet and legs	0.07	0.40			•	
	mammary system	0.13	0.47			•	
	fore udder	0.11	0.45			•	
	rear udder	0.08	0.36			•	
	milk yield	0.04				•	vanRaden et al. 1991
	milk fat content	0.03			•		
Jersey cattle	stature	0.05	0.12			•	Thomas et al. 1985
	strength	0.10	0.29			•	
	dairy character	0.71	0.81		•		
	foot angle	0.14	0.56			•	
	rear legs	0.01	0.07			•	
	rump angle	0.28	0.48			•	
	rump width	0.45	0.68			•	
	fore udder	0.60	0.79			•	
	rear udder height	0.55	0.78			•	
	rear udder width	0.49	0.75			•	
	udder depth	0.14	0.35			•	
	suspensory ligament	0.38	0.72			•	
	teat placement	0.20	0.55			•	
Lablab purpureus (bean)	plant height		0.72			•	Ushakumari and Chandrasekharan 1992
	stem girth		0.50			•	
	# of primaries		0.96			•	
	# of secondaries		0.86			•	
	# of leaves		0.63			•	
	leaf area		0.89			•	
	green fodder yield		0.76			•	
	dry weight of leaf		0.73			•	
	dry weight of stem		0.71			•	
	dry weight/plant		0.77			•	
	dry matter production		0.76			•	

	carotene content		0.59		•		
	NO_3 content		0.50		•		
	phosphorus content		0.50		•		
	crude protein		0.74		•		
	potassium content		0.94		•		
	calcium content		0.83		•		
Lycopersicon esculentum (tomato)	germination time	0.00	0.00	•			Fooland and Jones 1991
	generation mean	0.17	0.45	•			Kozik et al. 1991
	fruit sugar accumulation		0.50		•		Stommel and Haynes 1993
	glucose:fructose ratio		0.00		•		
Oryctolagus cuniculus (rabbit)	body weight	0.55	0.57			•	Lukefahr et al. 1992
Oryza sativa (rice)	protein content	0.63	0.47		•		Shenoy et al. 1991
	tiller number		0.49			•	Xu and Shen 1991
Rye	plant height		0.58			•	Izdebski 1992
	spike length		0.17			•	
	peduncle length		0.24			•	
Saccharum spp (sugar-cane)	brix value	0.27	0.33		•		Hogarth 1971
	weight/stool	0.05	0.22			•	
	stalks/stool	0.00	0.00			•	
	weight/stalk	0.00	0.00			•	
	grade	0.14	0.44			•	
	arrowing percentage		0.36			•	
	brix value		0.23		•		Hogarth 1977
	cane yield		0.59			•	
	weight/stalk		0.08			•	
	refractometer solids		0.24		•		Hogarth et al. 1981
	stalk number		0.42			•	
	stalk diameter		0.29			•	
	stalk length		0.31			•	
	volume of stool		0.50			•	
Sesame	resistance to charcoal rot		0.92		•		Sinhamahapatra and Das 1992
Sugarbeet	bolting resistance		0.08	•			Jolliffe and Arthur 1993
Wheat	plant height		0.25			•	Singh 1990
	tiller number		0.37			•	
	grains/ear		0.37			•	
	grain weight		0.37			•	
	yield/plant		0.34	•			
Zea mays (corn)	resistance to head smut		0.11		•		Bernardo et al. 1992
	resistance to corn leaf aphid		1.00		•		Bing and Guthrie 1991
	osmotic adjustment	0.32	0.75		•		Guei and Wassom 1993
	grain yield		0.26	•			Keeratinijakal and Lamkey 1993
	grain moisture		0.00		•		
	root lodging		0.26			•	
	stalk lodging		0.81			•	
	ear height		0.24			•	
	plant height		0.17			•	

silking date	0.00	•			
pollen date	0.00	•			
phosphorus concentration	0.45		•		Rakha et al. 1992
yield	0.36	•			Williams et al. 1965
ear length	0.41			•	
ear diameter	0.04			•	
number of kernel rows	0.03			•	
weight/100 kernels	0.12			•	
grain yield/plant	0.57	•			Zargar and Singh 1990
days to silk	0.45	•			
plant height	0.37			•	
ear height	0.40			•	
ear length	0.24			•	
ear diameter	0.67			•	
kernel rows/ear	0.35			•	
100-kernel weight	0.40			•	

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Appendix 2. A list of species, trait type, outbred fitness, inbred fitness values and calculated levels of inbreeding depression for birds, mammals, poikilotherms and plants measured in the wild. Note: $\delta = 1 - \frac{\text{inbred}}{\text{outbred}}$; Art. Inbred=indicates which species were artificially inbred; Sig.?=indicates which estimates of outbred fitness values are significantly greater than inbred fitness values; *=indicates that the trait type was inappropriate for the analysis (outbred<inbred) and could not be modified; **=indicates that the trait was modified from outbred<inbred to oubred>inbred for the analysis.

Species	Trait	Outbred	Inbred	F	|δ|	Art. Inbred	Sig ?	Reference
ANIMALS								
Birds								
Accipter cooperii	clutch size	4	3.7		0.075	no	?	Rosenfield & Bielefeldt 1992.
Geospiza fortis	clutch size	3.49, 3.57	3.50, 3.34	0.085, 0.125	0.031	no	no	Gibbs & Grant 1989
	# eggs hatched	2.01, 1.92	1.65, 1.68	0.085, 0.125	0.152		no	
	# young fledged	1.67, 1.58	1.40, 1.52	0.085, 0.125	0.1		no	
	# young surviving	0.69, 0.77	0.52, 0.72	0.085, 0.125	0.155		no	
	% eggs hatched	54, 51	45, 44	0.085, 0.125	0.152		no	
	% young fledged	79, 79	85, 75	0.085, 0.125	-0.013		no	
	% eggs fledged	45, 41	38, 36	0.085, 0.125	0.139		no	
	% young surviving	48, 51	33, 39	0.085, 0.125	0.274		no	
Geospiza magnirostris	hatching success	0.84	0.73	0.092	0.173	no	no	Grant & Grant 1995
	fluctuating asymetry	-0.068	2.33	0.092	?		yes	
Malurus splendens	number of nests	127	47		0.63	no	?	Rowley et al. 1986
	number of eggs	378	142		0.62		?	
	number of nestlings	336	125		0.63		?	
	number of fledglings	301	109		0.64		?	
	number of yearlings	115	46		0.6		?	
	clutch size	3	3		0		no	
	clutches/year/female	1.7	1.8		-0.06		no	
	nestlings/eggs	0.89	0.88		0.01		?	
	fledglings/nestlings	0.9	0.87		0.03		?	
	yearlings/fledglings	0.38	0.42		-0.11		no	
	nesting survival	79.7	76.8		0.04		no	
Melospiza melodia	reproductive success	0.13	-0.18		?	no	no	Arcese 1989
Melospiza melodia	survival	135, 95	30, 5		0.863	no	yes	Keller et al. 1994
Parus major	% fledging survival	82.5	62		0.248	no	?	Bulmer 1973
	% survival after fledging	6.8	0		1		yes	
Parus major	clutch size	8.38	8		0.045	no	no	Greenwood et al. 1978

	% nestling survival**	83 8	72 3		0 137		yes	
	% fledging recovery	10	5 8		0 42		no	
Parus major	hatching success I**	0 963	0 8		0 169	no	yes	van Noordwijk & Scharloo 1981
	hatching success II**	0 851	0 679		0 202		yes	
	brood success**	0.779	0 634		0.186		no	
	fledging survival	14.5	16		-0 103		no	
Mammals								
Acinonyx jubatus	juvenile survivorship**	73 7	55 8		0 243	no	?	O'Brien et al. 1985
Cynomys ludovicianus	probability producing litter	0.51	0 5		0 02	no	no	Hoogland 1992
	litter size	3 16	3		0 051		no	
	juvenile weight	152	148		0 026		no	
	# emergent young	1 53	1.33		0.131		no	
	% emergent young	49	67		-0 367		no	
Leontopithecus rosalia	# offspring surviving	0.829	0.474		0.428	no	yes	Dietz & Baker 1993
	% reproductive success	86	0		1		yes	
Panthera leo	ejaculate volume	9.4	5.9		0.372	no	yes	Wildt et al. 1987
	sperm motility	91	61		0.33		yes	
	sperm per ejaculate	34.4	13 3		0 613		no	
	motile sperm/ejaculate	228 5	45 3		0 802		yes	
	total sperm abnormalities*	24 8	66 2		-1 669		yes	
Papio anubis	% offspring viability	84 2	50		0 406	no	yes	Packer 1979
Papio cynocephalus ursinus	% infant survival/30d.	4	0		1	no	no	Bulger & Hamilton 1988
Papio cynocephalus	% offspring survival	81	0		1	no	yes	Alberts & Altmann 1995
Peromyscus leucopus nov.	% survivorship**	100	56	0 25	0 44	yes	yes	Jimenez et al. 1994
	body mass	21.4	21 8	0 25	-0 019		yes	
Sorex araneus	offspring survival	0 35	0 28		0 2	no	yes	Stockley et al. 1993

Poikilotherms								
Ananta arbustorum	# of clutches	17	13.6	0.25	0.2	yes	no	Chen 1993
	clutch size	19.7	17.2	0.25	0.127		no	
	# of eggs	313.9	244.6	0.25	0.221		no	
	hatching success	0.485	0.304	0.25	0.373		yes	
	# hatchlings	164.6	73	0.25	0.557		yes	
	# dead embryos*	40	22.14	0.25	0.447		no	
	reproductive success**	0.5	0.33	0.25	0.34		yes	
	proportion surviving	0.8	0.58	0.25	0.275		yes	
Salmo gairdneri	% survival of eyed eggs	95.1, 94.6, 96.8	86.2, 79.3, 91.0	0.25, 0.375, 0.5	0.1	yes	yes	Gjerde et al. 1983
	% survival of alevins	99.0, 98.4, 96.3	96.2, 90.1, 91.5	0.25, 0.375, 0.5	0.053		yes	
	% survival of fry	81.4, 72.7, 72.6	72.3, 67.1, 54.0	0.25, 0.375, 0.5	0.111		yes	
	growth of fingerlings	12.0, 47.8, 12.2	10.8, 37.0, 12.7	0.25, 0.375, 0.5	0.094		no	
	growth of adults	2.50, 3.15, 2.96	2.22, 2.52, 2.05	0.25, 0.375, 0.5	0.21		no	
Salmo salar	recapture frequency	0.052	-0.418	0.25	?	yes	yes	Ryman 1970
Vipera berus	brood size**	10	7		0.3	no	yes	Madsen et al. 1996
	% viable offspring**	91	68.4		0.248		yes	
PLANTS								
Chamaecrista fasciculata	% fruit maturation	0.5	0.45		0.1	yes	no	Fenster 1991
	% seed germination	0.35	0.3		0.143		no	
	flower production/plant	8	4		0.5		yes	
	# seeds/fruit	10.5	9.4		0.105		yes	
	% survival	0.44	0.3		0.318		no	
	fruit production/plant	1.6	0.65		0.594		no	
	progeny fitness	0.6	0.13		0.783		yes	
Costus allenii	seed production	46.7	30.5	1	0.35	yes	yes	Schemske 1983
	# seeds germinated	96, 9	94, 10	1, 1	-0.04		no	
	# seedlings surviving	10, 0	12, 0	1, 1	-0.1		no	
Costus laevis	seed production	52.9	26.2	1	0.5	yes	yes	Schemske 1983
	# seeds germinated	130, 10	109, 11	1, 1	0.03		no	

	# seedlings surviving	12, 0	11, 0	1, 1	0 04		no	
Costus guanaiensis	seed production	107 9	81 2	1	0 25	yes	yes	Schemske 1983
Delphinium nelsoni	seeds per flower	15, 16, 10, 13	10, 9, 5, 0	1, 1, 1, 1	0.573	yes	yes	Price & Waser 1979
	seedling survival	0.159, 0.071	0 052, 0	1, 1	0.837		yes	
Erythronium americanum	% fruit production	75	33 3	1	0 556	yes	yes	Harder et al. 1985
	seed production	41.2	10.5	1	0.745		yes	
	average seed mass	3 6	3 8	1	-0 056		no	
Gilia achilleifolia	% seeds not aborted**	96 9	97.1		-0 002	yes	no	Schoen 1983
	% seed germination	55 4	85 7		-0 55		yes	
	% seedling establishment	100	69		0.31		yes	
	% survival	0.5	0.25		0.5		yes	
	average seed production	6 69	7.53		-0.126		no	
	# capsules per plant	5 51	4.18		0 241		no	
	relative fitness	1	0 57		0 43		yes	
Ipomopsis aggregata	seed size	1.58, 1.50	1.44, 1 21		0.141	yes	yes	Heschel & Paige 1995
	germination success	38 32, 38 57	27 68, 22 68		0.342		yes	
	% survival**	87.5	71 2		0.186		yes	
	final height	27.4	23 6		0.139		yes	
Limnanthes alba	# flowers per plant	58 3, 58 2, 63	50.1, 50 9, 58 8		0.11	no	yes	Jain 1978
	plant height	22.1, 20 7, 19 9	18 3, 18 5, 18.7		0.11		yes	
	seed per flower	1 08, 0 9, 0.77	0 50, 0 53, 0 54		0 42		yes	
Lobelia cardinalis	survival**	100	54		0.46	yes	no	Johnston 1992
	flower number**	100	55		0 45		no	
	% flowering**	100	47		0 53		yes	
	net fertility**	100	29		0 71		yes	
Lobelia spicata	flower number**	100	71		0 29	yes	yes	Johnston 1992
	net fertility**	100	46		0 54		yes	
Lolium multiflorum	heading date*	15 56	18 32		-0 177	yes	yes	Polans Allard 1989

	tiller height	108 27	91 96		0 151		yes	
	# spikelets	38 8	33 85		0 128		yes	
	glume length	12 52	11 22		0.104		yes	
	# tillers	220 22	173 59		0 212		yes	
	seed weight	2 9	2 88		0 007		yes	
Sabatia angularis	% progeny survival	68	50	1	0 265	yes	yes	Dudash 1990
	total fruit mass	98	40	1	0 592		yes	
	relative progeny fitness	99	25	1	0 747		yes	
	seed number per fruit	834	730	1	0 125		yes	
	seed mass/fruit	0 023	0 022	1	0 043		no	
Sidalcea oregana	% seeds germinated	14 01	13 47	1	0 039	yes	no	Ashman 1992
	juvenile growth rate	0 0277	0 0324	1	-0 17		no	
	% seedling survival	67	59	1	0 119		no	
	% offspring reproductive	49 5	46 5	1	0 061		no	
	plant size	140	80	1	0 429		yes	
	# flowers produced	54	40	1	0 259		yes	
	multiplicative fitness	0 074	0 06	1	0 189		no	
Zostera marina	seed set	0 437, 0 273	0 306, 0 217	1, 1	0 253	yes	yes	Ruckelshaus 1995
	mean fitness	1 0, 1 0	0 7, 0 795	1, 1	0 253		yes	

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Appendix 3. Raw data for the three experiments using *Gryllus firmus* males. Where: wing morph: 0=LW, 1=SW; pair #: indicates pair designation of each male; call dur.: call duration in hours; DLM wt.: dorsal longitudinal muscle weight (g); DLM condition: dorsal longitudinal muscle condition: 0=white, 1=beige/pink, 2=brick red; female: whether or not a male attracted a female in a T-maze; cage #: cage designation of females for quantitative genetic experiment; treatment: 0=control, 1=resource restricted.

Experiment 1

	wing morph	pair #	call dur. (h)	dry body wt.	DLM wt.	DLM condition	lipid wt.	female
1	0.0	1.0	0.0550	0.1220	0.0075	1.0	0.0322	no
2	0.0	2.0	0.7430	0.1324	0.0051	1.0	0.0612	.
3	0.0	3.0	0.8940	0.1134	0.0063	1.0	0.0536	no
4	0.0	4.0	0.1740	0.1256	0.0091	2.0	0.0211	yes
5	0.0	5.0	0.0220	0.1222	0.0101	2.0	0.0113	no
6	0.0	6.0	1.2900	0.1433	0.0032	0.0	0.0679	yes
7	0.0	7.0	1.9840	0.0923	0.0012	0.0	0.0621	.
8	0.0	8.0	1.1110	0.1342	0.0064	2.0	0.0568	no
9	0.0	9.0	0.2880	0.1300	0.0070	2.0	0.0357	no
10	0.0	10.0	0.1570	0.1287	0.0034	0.0	0.0216	yes
11	0.0	11.0	1.2320	0.1177	0.0067	2.0	0.0522	no
12	0.0	12.0	0.2660	0.1290	0.0078	2.0	0.0432	no
13	0.0	13.0	0.7090	0.1178	0.0043	2.0	0.0418	no
14	0.0	14.0	0.7250	0.1109	0.0053	2.0	0.0217	yes
15	0.0	15.0	0.1600	0.1432	0.0074	2.0	0.0116	no
16	0.0	16.0	0.6290	0.1188	0.0021	0.0	0.0387	no
17	0.0	17.0	0.3660	0.1256	0.0011	0.0	0.0227	no
18	0.0	18.0	0.2100	0.1300	0.0084	1.0	0.0467	yes
19	0.0	19.0	0.5870	0.1249	0.0048	1.0	0.0122	no
20	0.0	20.0	0.3340	0.1221	0.0072	2.0	0.0098	no
21	0.0	21.0	0.4200	0.1267	0.0038	0.0	0.0218	no
22	0.0	22.0	0.5300	0.1203	0.0058	2.0	0.0542	no
23	1.0	1.0	2.6020	0.1278	0.0000		0.0762	yes
24	1.0	2.0	2.0190	0.1243	0.0000		0.0875	.
25	1.0	3.0	0.5260	0.1387	0.0000		0.0568	yes
26	1.0	4.0	2.8690	0.1322	0.0000		0.0765	no
27	1.0	5.0	0.7420	0.1300	0.0000		0.0452	yes
28	1.0	6.0	0.2690	0.1176	0.0000		0.0358	no
29	1.0	7.0	0.7470	0.1121	0.0000		0.0421	.
30	1.0	8.0	0.9200	0.1276	0.0000		0.0327	yes
31	1.0	9.0	2.2010	0.1294	0.0000		0.0564	yes
32	1.0	10.0	0.5390	0.1238	0.0000		0.0321	no
33	1.0	11.0	1.0780	0.1287	0.0000		0.0433	yes
34	1.0	12.0	0.4840	0.1232	0.0000		0.0210	yes
35	1.0	13.0	1.0690	0.1304	0.0000		0.0121	yes
36	1.0	14.0	1.8300	0.1226	0.0000		0.0165	no
37	1.0	15.0	0.8090	0.1298	0.0000		0.0231	yes
38	1.0	16.0	1.2900	0.1145	0.0000		0.0178	yes
39	1.0	17.0	0.9460	0.0945	0.0000		0.0218	yes
40	1.0	18.0	0.5030	0.0921	0.0000		0.0432	no
41	1.0	19.0	0.6790	0.1167	0.0000		0.0463	yes
42	1.0	20.0	0.5590	0.1176	0.0000		0.0671	yes
43	1.0	21.0	0.3650	0.1254	0.0000		0.0219	yes
44	1.0	22.0	0.9990	0.1156	0.0000		0.0352	yes

Experiment 2

	wing morph	pair #	cage #	call dur. (h)	dry body wt.	DLM wt.	DLM condition	lipid wt.	female
1	0.0	1.0	1.0	0.5860	0.1270	0.0086	2.0	0.0690	no
2	0.0	2.0	1.0	0.5260	0.1290	0.0056	2.0	0.0531	no
3	0.0	3.0	2.0	0.5640	0.1290	0.0053	1.0	0.0531	no
4	0.0	4.0	2.0	0.6510	0.1120	0.0025	2.0	0.0429	yes
5	0.0	5.0	2.0	0.7640	0.0920	0.0063	2.0	0.0300	yes
6	0.0	6.0	2.0	0.8620	0.1440	0.0081	0.0	0.0539	no
7	0.0	7.0	2.0	0.7990	0.1270	0.0002	0.0	0.0321	yes
8	0.0	8.0	2.0	0.7710	0.1190	0.0031	1.0	0.0429	no
9	0.0	9.0	1.0	0.6920	0.1140	0.0096	2.0	0.0602	no
10	0.0	10.0	1.0	0.7960	0.1180	0.0027	0.0	0.0401	no
11	0.0	11.0	1.0	0.5320	0.1130	0.0095	2.0	0.0361	yes
12	0.0	12.0	2.0	0.5980	0.1240	0.0091	2.0	0.0480	no
13	0.0	13.0	2.0	0.4990	0.1280	0.0088	2.0	0.0440	no
14	0.0	14.0	1.0	0.2140	0.1360	0.0092	2.0	0.0591	no
15	0.0	15.0	1.0	0.5600	0.1180	0.0092	2.0	0.0530	no
16	0.0	16.0	1.0	0.7190	0.1140	0.0048	2.0	0.0789	no
17	0.0	17.0	1.0	0.8110	0.1140	0.0046	2.0	0.0561	yes
18	0.0	18.0	1.0	0.6770	0.1170	0.0048	1.0	0.0421	no
19	0.0	19.0	2.0	0.7050	0.1300	0.0053	2.0	0.0529	no
20	0.0	20.0	2.0	0.7300	0.1320	0.0053	2.0	0.0519	no
21	0.0	21.0	1.0	0.5970	0.1160	0.0095	2.0	0.0431	no
22	0.0	22.0	2.0	0.5070	0.1130	0.0094	2.0	0.0460	no
23	0.0	23.0	1.0	0.5120	0.1550	0.0100	2.0	0.0541	no
24	0.0	24.0	1.0	0.5600	0.1460	0.0091	2.0	0.0652	no
25	0.0	25.0	2.0	0.5860	0.1430	0.0088	2.0	0.0571	no
26	0.0	26.0	1.0	0.5610	0.1350	0.0054	2.0	0.0520	no
27	0.0	27.0	1.0	0.4770	0.1230	0.0139	2.0	0.0762	no
28	0.0	28.0	2.0	0.6370	0.1370	0.0081	2.0	0.0221	no
29	0.0	29.0	2.0	0.5970	0.1380	0.0023	1.0	0.0230	no
30	0.0	30.0	2.0	0.5260	0.1310	0.0044	2.0	0.0430	yes
31	0.0	31.0	1.0	0.5260	0.1170	0.0005	0.0	0.0341	no
32	0.0	32.0	1.0	0.5530	0.0970	0.0056	0.0	0.0431	no
33	0.0	33.0	2.0	0.7880	0.0930	0.0113	1.0	0.0210	no
34	0.0	34.0	2.0	0.7570	0.0850	0.0009	1.0	0.0451	no
35	0.0	35.0	2.0	0.5990	0.0820	0.0064	0.0	0.0369	yes
36	0.0	36.0	2.0	0.5720	0.1120	0.0034	1.0	0.0480	yes
37	0.0	37.0	1.0	0.5860	0.1260	0.0069	2.0	0.0380	no
38	0.0	38.0	2.0	0.5530	0.1380	0.0091	2.0	0.0519	no
39	0.0	39.0	2.0	0.5990	0.1290	0.0046	2.0	0.0559	yes
40	0.0	40.0	1.0	0.6120	0.1270	0.0058	2.0	0.0341	no
41	0.0	41.0	1.0	0.5240	0.1280	0.0069	2.0	0.0019	no
42	0.0	42.0	1.0	0.5770	0.1240	0.0066	2.0	0.0340	no
43	0.0	43.0	2.0	0.5300	0.1600	0.0068	2.0	0.0660	no
44	0.0	44.0	2.0	0.6400	0.1310	0.0066	2.0	0.0620	no
45	0.0	45.0	2.0	0.5970	0.1320	0.0064	2.0	0.0511	no

Experiment 2

	wing morph	pair #	cage #	call dur. (h)	dry body wt.	DLM wt.	DLM condition	lipid wt.	female
46	0.0	46.0	2.0	0.5300	0.1200	0.0078	2.0	0.0580	no
47	0.0	47.0	1.0	0.5680	0.1300	0.0082	2.0	0.0691	no
48	0.0	48.0	2.0	0.6400	0.1250	0.0073	2.0	0.0787	yes
49	0.0	49.0	1.0	0.5950	0.1370	0.0075	2.0	0.0340	no
50	0.0	50.0	2.0	0.6060	0.1300	0.0071	2.0	0.0568	no
51	0.0	51.0	1.0	0.6250	0.1130	0.0072	2.0	0.0341	no
52	0.0	52.0	2.0	0.5840	0.1170	0.0063	2.0	0.0450	yes
53	0.0	53.0	2.0	0.6140	0.1200	0.0043	1.0	0.0411	no
54	0.0	54.0	2.0	0.5870	0.1230	0.0067	2.0	0.0671	no
55	0.0	55.0	1.0	0.5850	0.1140	0.0074	2.0	0.0539	no
56	0.0	56.0	1.0	0.6120	0.1150	0.0053	2.0	0.0549	no
57	0.0	57.0	2.0	0.6370	0.1180	0.0089	2.0	0.0381	yes
58	0.0	58.0	2.0	0.6060	0.1120	0.0042	2.0	0.0570	no
59	0.0	59.0	1.0	0.6100	0.1010	0.0060	2.0	0.0229	yes
60	0.0	60.0	1.0	0.5170	0.0790	0.0063	2.0	0.0280	no
61	0.0	61.0	1.0	0.8110	0.1150	0.0042	1.0	0.0541	yes
62	0.0	62.0	2.0	0.7710	0.1360	0.0007	0.0	0.0500	yes
63	0.0	63.0	1.0	0.6370	0.1490	0.0036	1.0	0.0613	no
64	0.0	64.0	1.0	0.6830	0.1420	0.0042	0.0	0.0669	no
65	0.0	65.0	2.0	0.6650	0.1290	0.0026	0.0	0.0430	no
66	0.0	66.0	1.0		0.0940	0.0090	2.0	0.0440	no
67	0.0	67.0	2.0	0.6650	0.0960	0.0041	2.0	0.0421	no
68	0.0	68.0	2.0	0.6270	0.1360	0.0064	2.0	0.0251	yes
69	0.0	69.0	1.0	0.6110	0.1390	0.0067	1.0	0.0581	no
70	0.0	70.0	1.0	0.5220	0.0970	0.0037	2.0	0.0491	no
71	0.0	71.0	1.0	0.5870	0.1370	0.0044	1.0	0.0551	yes
72	0.0	72.0	1.0	0.5750	0.1370	0.0073	2.0	0.0459	yes
73	0.0	73.0	2.0	0.5280	0.1390	0.0041	2.0	0.0579	no
74	0.0	74.0	2.0	0.6510	0.1310	0.0022	0.0	0.0555	yes
75	0.0	75.0	2.0	0.7130	0.1290	0.0089	2.0	0.0451	yes
76	0.0	76.0	1.0	0.9220	0.1220	0.0000	0.0	0.0540	yes
77	0.0	77.0	2.0	0.7710	0.1350	0.0096	1.0	0.0340	no
78	0.0	78.0	1.0	0.5230	0.1190	0.0035	1.0	0.0459	no
79	0.0	79.0	2.0	0.9860	0.1540	0.0065	0.0	0.0689	yes
80	0.0	80.0	1.0	0.5600	0.1490	0.0016	0.0	0.0781	no
81	0.0	81.0	2.0	0.5260	0.1260	0.0096	1.0	0.0020	no
82	0.0	82.0	2.0	0.6500	0.1290	0.0003	0.0	0.0010	no
83	0.0	83.0	1.0	0.8530	0.1200	0.0044	0.0	0.0571	yes
84	0.0	84.0	1.0	0.7720	0.1200	0.0035	0.0	0.0340	yes
85	0.0	85.0	1.0	0.9330	0.1540	0.0118	1.0	0.0419	yes
86	0.0	86.0	1.0	0.5310	0.0930	0.0091	2.0	0.0210	no
87	0.0	87.0	2.0	0.5970	0.0920	0.0088	2.0	0.0110	no
88	0.0	88.0	2.0	0.5600	0.0960	0.0087	2.0	0.0341	yes
89	0.0	89.0	2.0	0.4910	0.1160	0.0082	2.0	0.0120	yes
90	0.0	90.0	1.0	0.4690	0.1190	0.0089	2.0	0.0179	yes

Experiment 2

	wing morph	pair #	cage #	call dur. (h)	dry body wt.	DLM wt.	DLM condition	lipid wt.	female
91	0.0	91.0	1.0	0.5860	0.1290	0.0042	1.0	0.0020	no
92	0.0	92.0	2.0	0.5590	0.1340	0.0072	2.0	0.0009	no
93	0.0	93.0	2.0	0.5250	0.1270	0.0034	2.0	0.0030	no
94	0.0	94.0	1.0	0.6510	0.1320	0.0037	1.0	0.0120	yes
95	0.0	95.0	1.0	0.7560	0.1300	0.0091	2.0	0.0171	yes
96	0.0	96.0	1.0	0.7700	0.1130	0.0075	0.0	0.0891	no
97	0.0	97.0	1.0	0.9130	0.0990	0.0001	0.0	0.0030	yes
98	0.0	98.0	2.0	0.6470	0.1240	0.0034	1.0	0.1339	yes
99	0.0	99.0	2.0	0.5970	0.1340	0.0152	1.0	0.0110	yes
100	0.0	100.0	1.0	0.7020	0.1170	0.0025	2.0	0.0200	no
101	0.0	101.0	1.0	0.7690	0.1000	0.0020	0.0	0.0020	yes
102	0.0	102.0	2.0	1.1190	0.1000	0.0031	1.0	0.0339	yes
103	0.0	103.0	1.0	0.7430	0.1120	0.0000	0.0	0.0210	no
104	0.0	104.0	1.0	0.6480	0.1350	0.0000	0.0	0.0010	no
105	0.0	105.0	2.0	1.0490	0.1420	0.0026	1.0	0.1210	yes
106	0.0	106.0	2.0	0.5970	0.0960	0.0079	2.0	0.0791	yes
107	0.0	107.0	2.0	0.7020	0.0920	0.0082	2.0	0.0910	yes
108	0.0	108.0	1.0	0.5170	0.1240	0.0054	2.0	0.0010	no
109	0.0	109.0	1.0	0.5960	0.1320	0.0078	2.0	0.0020	yes
110	0.0	110.0	1.0	0.5070	0.1250	0.0089	2.0	0.0560	no
111	1.0	1.0	1.0	0.9320	0.1450	0.0000		0.0480	yes
112	1.0	2.0	1.0	0.8600	0.1570	0.0000		0.0520	yes
113	1.0	3.0	2.0	0.9250	0.1260	0.0000		0.0570	yes
114	1.0	4.0	2.0	0.9580	0.1380	0.0000		0.3000	no
115	1.0	5.0	2.0	0.9020	0.1480	0.0000		0.4900	no
116	1.0	6.0	2.0	1.0920	0.1330	0.0000		0.0410	yes
117	1.0	7.0	2.0	0.6400	0.1340	0.0000		0.0440	yes
118	1.0	8.0	2.0	0.7960	0.1220	0.0000		0.0380	yes
119	1.0	9.0	1.0	0.9940	0.1380	0.0000		0.0010	no
120	1.0	10.0	1.0	0.7220	0.0760	0.0000		0.0440	yes
121	1.0	11.0	1.0	0.7990	0.0990	0.0000		0.0370	no
122	1.0	12.0	2.0	0.7710	0.0860	0.0000		0.0390	yes
123	1.0	13.0	2.0	0.9060	0.1220	0.0000		0.2800	yes
124	1.0	14.0	1.0	0.8870	0.1380	0.0000		0.4000	yes
125	1.0	15.0	1.0	0.7950	0.1460	0.0000		0.0410	yes
126	1.0	16.0	1.0	0.7570	0.1500	0.0000		0.0260	yes
127	1.0	17.0	1.0	0.6320	0.1150	0.0000		0.0230	no
128	1.0	18.0	1.0	0.8530	0.1230	0.0000		0.0310	yes
129	1.0	19.0	2.0	0.7310	0.1290	0.0000		0.0210	yes
130	1.0	20.0	2.0	0.8240	0.1150	0.0000		0.0180	yes
131	1.0	21.0	1.0	0.9060	0.1280	0.0000		0.0550	yes
132	1.0	22.0	2.0	0.9760	0.1310	0.0000		0.0580	yes
133	1.0	23.0	1.0	0.9580	0.1190	0.0000		0.0430	yes
134	1.0	24.0	1.0	0.9870	0.1180	0.0000		0.0440	yes
135	1.0	25.0	2.0	0.9660	0.1170	0.0000		0.0520	yes

Experiment 2

	wing morph	pair #	cage #	call dur. (h)	dry body wt.	DLM wt.	DLM condition	lipid wt.	female
136	1.0	26.0	1.0	1.0500	0.1460	0.0000		0.0630	yes
137	1.0	27.0	1.0	1.1180	0.1390	0.0000		0.0660	yes
138	1.0	28.0	2.0	0.9830	0.1350	0.0000		0.0780	yes
139	1.0	29.0	2.0	0.9990	0.1300	0.0000		0.0480	yes
140	1.0	30.0	2.0	1.0140	0.1450	0.0000		0.0560	no
141	1.0	31.0	1.0	0.7840	0.1330	0.0000		0.0500	yes
142	1.0	32.0	1.0	1.1200	0.1350	0.0000		0.0010	yes
143	1.0	33.0	2.0	0.5260	0.1330	0.0000		0.0430	no
144	1.0	34.0	2.0	0.6490	0.1300	0.0000		0.0420	yes
145	1.0	35.0	2.0	0.9830	0.1280	0.0000		0.0560	yes
146	1.0	36.0	2.0	0.8120	0.1240	0.0000		0.0270	no
147	1.0	37.0	1.0	0.8380	0.1280	0.0000		0.0310	yes
148	1.0	38.0	2.0	0.7700	0.1470	0.0000		0.0370	yes
149	1.0	39.0	2.0	0.7350	0.1390	0.0000		0.0270	no
150	1.0	40.0	1.0	0.7840	0.1570	0.0000		0.0400	yes
151	1.0	41.0	1.0	0.9850	0.1380	0.0000		0.0580	yes
152	1.0	42.0	1.0	0.9710	0.1330	0.0000		0.0610	yes
153	1.0	43.0	2.0	1.0920	0.1290	0.0000		0.0680	yes
154	1.0	44.0	2.0	1.0650	0.1350	0.0000		0.0540	yes
155	1.0	45.0	2.0	1.0220	0.1360	0.0000		0.0470	yes
156	1.0	46.0	2.0	0.9530	0.1380	0.0000		0.0530	yes
157	1.0	47.0	1.0	0.9580	0.1220	0.0000		0.0030	yes
158	1.0	48.0	2.0	0.9860	0.1420	0.0000		0.0250	no
159	1.0	49.0	1.0	0.9420	0.1260	0.0000		0.0570	yes
160	1.0	50.0	2.0	0.9510	0.1380	0.0000		0.0670	yes
161	1.0	51.0	1.0	0.7990	0.1240	0.0000		0.0370	yes
162	1.0	52.0	2.0	0.7580	0.1520	0.0000		0.0320	no
163	1.0	53.0	2.0	0.8540	0.1220	0.0000		0.0330	yes
164	1.0	54.0	2.0	0.8270	0.1430	0.0000		0.0380	yes
165	1.0	55.0	1.0	0.9150	0.1220	0.0000		0.0210	yes
166	1.0	56.0	1.0	0.7830	0.1180	0.0000		0.0310	yes
167	1.0	57.0	2.0	0.7700	0.1490	0.0000		0.0200	no
168	1.0	58.0	2.0	0.8370	0.1550	0.0000		0.0190	yes
169	1.0	59.0	1.0	0.7350	0.1200	0.0000		0.0370	no
170	1.0	60.0	1.0	0.7560	0.1120	0.0000		0.0410	yes
171	1.0	61.0	1.0	0.8650	0.0820	0.0000		0.0440	no
172	1.0	62.0	2.0	0.7630	0.1140	0.0000		0.0320	no
173	1.0	63.0	1.0	0.9060	0.0920	0.0000		0.0350	yes
174	1.0	64.0	1.0	0.8530	0.0920	0.0000		0.0350	yes
175	1.0	65.0	2.0	0.8960	0.1120	0.0000		0.0460	yes
176	1.0	66.0	1.0		0.1250	0.0000		0.0350	yes
177	1.0	67.0	2.0	0.8890	0.1290	0.0000		0.0370	yes
178	1.0	68.0	2.0	0.8500	0.1130	0.0000		0.0320	no
179	1.0	69.0	1.0	0.8120	0.1110	0.0000		0.0310	yes
180	1.0	70.0	1.0	0.7700	0.0920	0.0000		0.0380	yes

Experiment 2

	wing morph	pair #	cage #	call dur. (h)	dry body wt.	DLM wt.	DLM condition	lipid wt.	female
181	1.0	71.0	1.0	0.5260	0.0930	0.0000		0.0130	no
182	1.0	72.0	1.0	0.6450	0.1270	0.0000		0.0160	no
183	1.0	73.0	2.0	0.8000	0.1280	0.0000		0.0210	yes
184	1.0	74.0	2.0	0.5000	0.1240	0.0000		0.0150	no
185	1.0	75.0	2.0	0.6480	0.1260	0.0000		0.0170	no
186	1.0	76.0	1.0	0.7720	0.1220	0.0000		0.4600	no
187	1.0	77.0	2.0	1.1200	0.1120	0.0000		0.0440	yes
188	1.0	78.0	1.0	1.0230	0.1500	0.0000		0.0400	yes
189	1.0	79.0	2.0	0.5950	0.1600	0.0000		0.0380	no
190	1.0	80.0	1.0	1.0670	0.1260	0.0000		0.0520	yes
191	1.0	81.0	2.0	0.7980	0.1400	0.0000		0.0330	yes
192	1.0	82.0	2.0	0.9590	0.1390	0.0000		0.0280	yes
193	1.0	83.0	1.0	1.1190	0.1470	0.0000		0.0290	no
194	1.0	84.0	1.0	0.5600	0.1300	0.0000		0.0340	no
195	1.0	85.0	1.0	0.5330	0.1230	0.0000		0.0300	no
196	1.0	86.0	1.0	0.7020	0.1390	0.0000		0.0200	yes
197	1.0	87.0	2.0	0.8260	0.1320	0.0000		0.0170	yes
198	1.0	88.0	2.0	0.6540	0.0930	0.0000		0.0190	no
199	1.0	89.0	2.0	0.5700	0.1130	0.0000		0.0230	no
200	1.0	90.0	1.0	0.5010	0.1290	0.0000		0.0300	no
201	1.0	91.0	1.0	0.8630	0.1200	0.0000		0.0400	yes
202	1.0	92.0	2.0	0.9660	0.1360	0.0000		0.0440	yes
203	1.0	93.0	2.0	0.8090	0.1570	0.0000		0.0370	yes
204	1.0	94.0	1.0	0.8600	0.1500	0.0000		0.0360	no
205	1.0	95.0	1.0	0.7150	0.1500	0.0000		0.0420	no
206	1.0	96.0	1.0	0.9290	0.1370	0.0000		0.0570	yes
207	1.0	97.0	1.0	0.8540	0.0980	0.0000		0.0550	no
208	1.0	98.0	2.0	1.0940	0.0790	0.0000		0.0670	no
209	1.0	99.0	2.0	1.0110	0.0990	0.0000		0.0620	no
210	1.0	100.0	1.0	1.0100	0.0850	0.0000		0.0450	yes
211	1.0	101.0	1.0	0.9100	0.1200	0.0000		0.0570	no
212	1.0	102.0	2.0	1.0230	0.1430	0.0000		0.0540	no
213	1.0	103.0	2.0	1.0950	0.1530	0.0000		0.0500	yes
214	1.0	104.0	1.0	0.9400	0.1380	0.0000		0.0020	yes
215	1.0	105.0	2.0	0.9590	0.1550	0.0000		0.0530	no
216	1.0	106.0	2.0	0.7990	0.1210	0.0000		0.0250	no
217	1.0	107.0	2.0	0.6490	0.1330	0.0000		0.0320	no
218	1.0	108.0	1.0	0.7560	0.1630	0.0000		0.0180	yes
219	1.0	109.0	1.0	0.7380	0.1310	0.0000		0.0090	no
220	1.0	110.0	1.0	0.9060	0.1320	0.0000		0.0230	yes

Experiment 3

	wing morph	pair #	total call (h)	dry body wt.	DLM wt.	DLM condition	lipid wt.	female	treatment
1	0.0	1.0	5.8090	0.1120	0.0000	0.0	0.0000	5	1.0
2	0.0	2.0	7.6630	0.0990	0.0000	0.0	0.0030	4	1.0
3	0.0	3.0	2.8940	0.0940	0.0000	0.0	0.0040	6	1.0
4	0.0	4.0	4.4470	0.1130	0.0000	0.0	0.0000	2	1.0
5	0.0	5.0	5.6990	0.1320	0.0011	1.0	0.0000	5	1.0
6	0.0	6.0	3.6470	0.1220	0.0010	0.0	0.0000	2	1.0
7	0.0	7.0	6.0060	0.1140	0.0020	1.0	0.0010	0	1.0
8	0.0	8.0	0.2460	0.0980	0.0000	0.0	0.0000	2	1.0
9	0.0	9.0	0.2130	0.0830	0.0000	0.0	0.0000	0	1.0
10	0.0	10.0	6.6280	0.1330	0.0000	0.0	0.0000	4	1.0
11	0.0	11.0	3.2710	0.1140	0.0010	0.0	0.0020	3	1.0
12	0.0	12.0	8.3060	0.1540	0.0000	0.0	0.0050	7	1.0
13	0.0	13.0	0.5670	0.0870	0.0010	0.0	0.0000	2	1.0
14	0.0	14.0	0.6990	0.0860	0.0030	1.0	0.0000	0	1.0
15	0.0	15.0	3.2760	0.1280	0.0000	0.0	0.0000	3	1.0
16	1.0	1.0	9.3490	0.1240	0.0000		0.0000	11	1.0
17	1.0	2.0	3.9670	0.1150	0.0000		0.0000	8	1.0
18	1.0	3.0	7.7370	0.1130	0.0000		0.0020	5	1.0
19	1.0	4.0	10.2360	0.1430	0.0000		0.0010	16	1.0
20	1.0	5.0	9.2990	0.1650	0.0000		0.0010	11	1.0
21	1.0	6.0	4.3050	0.1220	0.0000		0.0010	8	1.0
22	1.0	7.0	7.6530	0.1320	0.0000		0.0010	4	1.0
23	1.0	8.0	10.8770	0.1650	0.0000		0.0040	13	1.0
24	1.0	9.0	11.5700	0.1320	0.0000		0.0000	8	1.0
25	1.0	10.0	14.6190	0.1220	0.0000		0.0070	9	1.0
26	1.0	11.0	10.2200	0.1550	0.0000		0.0000	8	1.0
27	1.0	12.0	4.0960	0.1110	0.0000		0.0000	6	1.0
28	1.0	13.0	3.6780	0.0980	0.0000		0.0000	3	1.0
29	1.0	14.0	1.2390	0.0830	0.0000		0.0000	6	1.0
30	1.0	15.0	11.1140	0.1280	0.0000		0.0050	10	1.0
31	0.0		21.8550	0.1420	0.0000	0.0	0.0040	6	0.0
32	0.0		0.2240	0.0990	0.0000	0.0	0.0030	4	0.0
33	0.0		22.3730	0.1490	0.0020	0.0	0.0520	4	0.0
34	0.0		3.9590	0.1120	0.0000	0.0	0.0020	3	0.0
35	0.0		12.3970	0.1230	0.0010	1.0	0.0060	5	0.0
36	0.0		24.8990	0.1560	0.0020	1.0	0.0640	5	0.0
37	1.0		24.4160	0.1640	0.0000		0.0740	10	0.0
38	1.0		24.9450	0.1330	0.0000		0.0830	8	0.0
39	1.0		19.7960	0.1420	0.0000		0.0210	6	0.0
40	1.0		14.3700	0.1320	0.0000		0.0490	10	0.0
41	1.0		36.2000	0.1760	0.0000		0.0060	12	0.0
42	1.0		27.3450	0.1540	0.0000		0.0650	6	0.0