

Does a negative genetic correlation between wing morph and early fecundity imply a functional constraint in *Gryllus firmus*?

Gray Stirling,^{1*} Daphne J. Fairbairn,² Shane Jensen³ and Derek A. Roff¹

¹Department of Biology, McGill University, ²Department of Biology, Concordia University and

³Department of Mathematics and Statistics, McGill University, Montreal, Quebec, Canada

ABSTRACT

We tested the hypothesis that wing muscle size and triglyceride production mediate a life-history trade-off between wing morph (or flight capability) and early fecundity. Roff *et al.* recently selected for lower number of eggs laid in the first week by *Gryllus firmus* (sand cricket) and reported a correlated increase in long-winged (and presumably flight-capable) females, but did not test structural or energetic reproductive costs associated with flight capability. We used ovary weight of virgin crickets as an index of their early fecundity and dorsal longitudinal flight muscle (DLM) weight as a quantitative measure of flight muscle status. A preliminary experiment indicated that DLM weight is highly correlated with other measures of DLM status (colour, histolysis state, size). We used a haemolymph assay for acylglycerol concentration as an index of mobile triglyceride derivatives thought to provide energy for flight. Both DLM weight and acylglycerol concentration were negatively related to ovary weight of long-winged crickets, indicating wing muscle size and triglyceride concentrations mediate reproductive costs. In short-winged females, only DLM weight had a negative phenotypic relationship with ovary weight. The heritability of DLM weight and acylglycerol concentration are unknown, so we used the direction and size of standardized differences between base and selected populations to test whether DLM weight and acylglycerol concentration are negatively genetically correlated with ovary weight. Acylglycerol concentration and DLM weight increased in the population selected for low numbers of eggs laid within both wing morphs. Large standardized differences in short-winged females provide strong corroborative evidence of a genetic relationship between early fecundity and flight capability. These results indicate negative genetic correlations between wing morph and early fecundity reflect inherited variation in costs of early reproduction, related to physiological investment in flight capability (flight muscle size and triglyceride allocation), rather than being an arbitrary assumption of a life-history trade-off model.

Keywords: correlated response, flight capability, insects, life-history trade-off, phenotypic costs.

* Address all correspondence to Gray Stirling, Department of Biology, McGill University, 1205 Avenue Dr Penfield, Montreal, Quebec H3A 1B1, Canada. e-mail: stirling@bio1.lan.mcgill.ca
Consult the copyright statement on the inside front cover for non-commercial copying policies.

INTRODUCTION

Trade-offs are a central feature of life-history theory, but the direction and significance of their underlying relationships are often untested assumptions. Furthermore, there are several opinions on how these assumptions should be tested (Partridge, 1992; Reznick, 1992). Different levels of integration are often studied separately. Selection and breeding designs test genetic architecture (Reznick, 1992; Roff *et al.*, 1999). Alternatively, experiments may test or manipulate functional relationships (Partridge, 1992; Gustafsson *et al.*, 1994; Sinervo and Basolo, 1996; Zera *et al.*, 1998). However, some authors have argued that, to develop a more profound understanding of life-history trade-offs, we need to relate genetic architecture to the functional architecture (Riska, 1989; Houle, 1991), expressed in physiological relationships which mediate control of life-history phenotypes (Stearns, 1992). Different levels of a life-history trade-off (genetic, physiological, phenotypic) are each assumed to have the same relationship (i.e. negative), but this assumption may not always be correct (Charlesworth, 1990). Negative genetic correlations between flight capability related characters and early fecundity have been demonstrated in *Gryllus firmus* (sand cricket) and several factors are thought to structure the physiological relationships underlying this trade-off. However, consistency of the trade-off model has not been tested across different levels of integration.

A life-history trade-off between wing morph and early fecundity has been studied extensively in wing dimorphic insects (Roff and Fairbairn, 1991; Zera and Denno, 1997). Selection and breeding studies have demonstrated that wing length morph (long or macropterous and short or micropterous) and early fecundity co-evolve and that this genetic relationship covaries negatively in wing dimorphic crickets (Roff, 1986, 1994; Roff and Bradford, 1996; Roff *et al.*, 1997, 1999). At the same time, Roff *et al.* (1999) argue that differences between the response to selection on wing morph (Roff, 1994) and fecundity (Roff *et al.*, 1999) could be explained by different rates of histolysis (see Fairbairn and Roff, 1990) or a model that incorporates an effect due to competition for resources between flight muscles and gonads in the long-winged morph.

Allocation of resources for production of high-energy triglycerides is thought to have a negative effect on the early reproduction of long-winged crickets (Mole and Zera, 1993). Alternatively, the breakdown of wing muscles (histolysis) may enhance ovary development (Tanaka, 1993; Gomi *et al.*, 1995). Flight muscle respiration rates vary with flight muscle colour or size and higher respiration rates may impose energetic demands that have a negative effect on ovary development (Zera *et al.*, 1998). Short-winged females have under-developed, degraded or histolysed flight muscles (Roff, 1989; Roff and Fairbairn, 1991; Zera *et al.*, 1997) and a lower average concentration of triglycerides than long-winged females (Zera and Mole, 1994). This suggests the lower average reproduction of the long-winged morph (Roff, 1986, 1989; Roff and Fairbairn, 1991; Zera and Denno, 1997) is related to investment in high-energy triglycerides, which are thought to maintain flight-capable wing muscles (Mole and Zera, 1993; Zera *et al.*, 1994, 1998). However, flight muscle maintenance and ovary development may not initially compete for common resources, since flight muscles are largely formed before ovary development begins (Johnson, 1969; Mole and Zera, 1993) and flight muscle histolysis (breakdown) may precede ovary development (Johnson, 1969; Roff, 1986; Tanaka, 1993). The phenology of ovary development and wing muscle histolysis is not certain, although long-winged crickets with smaller, light coloured or histolysed flight muscles have early fecundities that are similar to those

of short-winged morphs (Roff, 1989; Tanaka, 1993; Zera *et al.*, 1997, 1998; Stirling *et al.*, 1999). On the one hand, this suggests flight muscle histolysis may enhance variability between long-winged morphs in the (physiological) cost of maintaining flight capability; on the other, nutrients derived from histolysing muscle may promote oogenesis (Tanaka, 1993; Gomi *et al.*, 1995), although there is little direct evidence for this relationship (Wheeler, 1996).

Negative associations between flight muscle size or condition and ovary mass of different wing morphs have long suggested a functional relationship governing the early fecundity–flight capability trade-off hypothesis (Roff, 1986; Zera and Denno, 1997). If triglyceride synthesis has a reproductive cost, then it should be highest in long-winged crickets that have not histolysed their wing muscles. We also expect that, if the functional relationships between muscle size or triglyceride concentration and ovary mass mediate genetic and phenotypic relationships, then they should have the same sign within wing morphs (i.e. negative). Roff *et al.* (1998) recently completed bi-directional selection on early fecundity of *Gryllus firmus* and reported significant bi-directional, correlated responses in wing morph proportion. This indicates there is a negative genetic correlation between wing morph liability (i.e. proportion long-winged) and a measure of early fecundity (i.e. lower number of eggs laid in the first week). If wing muscle size and triglyceride concentration are co-inherited with fecundity (genetically integrated; Cheverud, 1996), then directional changes in triglyceride concentrations or wing muscle size that are opposite to that of fecundity would provide evidence of negative genetic correlations. Alternatively, divergent character values in a selected population could be due to linkage disequilibria caused by repeated, strong selection, although this difference would rapidly decay (within four generations after selection is relaxed; eqn 4.11 in Roff, 1997). Flight capability or early fecundity character differences between base and selected populations may also be due to the difference in the proportion of macropterous females in each population, although Roff *et al.* (1999) found that mechanism only explained a small percentage of the change in fecundity. Finally, a between-population difference in rates of flight muscle histolysis could also account for changes in fecundity (Fairbairn and Roff, 1990; Roff *et al.*, 1999), either because different rates cause differences in average dorsal longitudinal flight muscle size, or because histolysis-derived nutrients promote ovary growth and development (Tanaka, 1993; Gomi *et al.*, 1995).

We first tested relationships between flight muscle status measures (size, colour, weight, condition) so that we could decide on the scale (categorical or continuous) to test the hypothetical functional trade-off with early fecundity.

METHODS

Preliminary experiment: Flight muscle status and weight

Dorsal longitudinal flight muscle. In the following studies, we only assayed the status of dorsal longitudinal flight muscles (DLM) rather than the entire thoracic flight muscle group (DLM and dorsoventral muscles; Zera *et al.*, 1997). Under standard conditions in the base population of *Gryllus firmus*, we had previously observed that, by the seventh day

after they eclosed to adults: (1) 95% of females had fully formed eggs in their ovaries; (2) approximately 50% of long-winged females had DLM with a visible degree of histolysis (partial or full histolysis), in contrast to the appearance of their dorsoventral muscles; (3) long-winged females that had partially or fully histolysed their DLM had ovary weights similar to those of short-winged females on average (Stirling *et al.*, 1999). This suggests that qualitative differences in DLM status are related to differences in early reproduction.

In the preliminary experiment, we assayed the following DLM characters to determine if four measures of DLM status (colour, histolysis condition, weight and size) are related. We collected 23 short-winged females over the first week after eclosion to adults and 36 long-winged females over the first 2 weeks (2 per day on days 1–7; 3–4 per day on days 8–14) in the base population.

DLM colour. Individuals were sacrificed in alcohol between 1 and 14 days after their last eclosion. Immediately afterwards, their metathorax was cut along the mid-dorsal line so that the DLM could be seen. The colour of the muscles was scored using ISCC-NBS colour charts. We used the red–pink scale and classified the colour of each DLM into one of five colour categories from white (1) to deep pink (5). Each individual was then placed in a vial filled with Bouins fluid.

DLM histolysis appearance. After preservation, each female was measured for head width and femur length. The dorsal mesothoracic plate was removed from each female and the DLM were visually classified on a 5-category scale. The DLM of females ranged from being well developed, large and firm (category 5), suggesting that they were intact, through a stage where the muscles appeared partly degraded (category 3) to DLM that were small, thin strips, with no apparent fibres (category 1). The last category includes females that had fully histolysed their DLM and those in which the DLM may never have developed.

DLM (wet) weight and volume. Either the right or left group of DLM was dissected out first, weighed and then measured in two directions (length and diameter). We estimated the volume of the DLM by assuming they were cylindrical in shape. This portion of the DLM was then fixed and mounted (described below). The other section of each individual's DLM was subsequently removed, weighed and then both weights were added together (DLM weight).

We examined sections of DLM microscopically to determine if the histolysis appearance category actually corresponded to DLM histolysis. The preserved tissue was first cleared in alcohol (30 min with each of 70%, 95% and 100% ethanol) and then put through three toluene washes (30 min each). The tissue was subsequently soaked in embedding wax for 3–4 h, mounted and then sectioned through the thickest portion of the muscle. The DLM from two long-winged and two short-winged crickets were selected on each day after eclosion, except day 1 where there was only one short-winged female. For the 11 individual DLMs sectioned up to day 3 after eclosion, six fibre diameter measurements were averaged as an estimate of an individual's fibre diameter (if the DLMs were intact) following Zera *et al.* (1997). The number of fibres was counted six times and then averaged to estimate the number of fibres in an individual's DLM cross-section.

Phenotypic relationships and population comparisons

Strong selection increases inbreeding. This process could produce significant line differences in characters which falsely suggest an association with the selected character (Henderson, 1997). To reduce inbreeding, we combined the two low fecundity replicates (Roff *et al.*, 1999) forming the 'selected' population and allowed this population to mate haphazardly for more than five generations. Roff *et al.* (1999) provide a detailed account of (1) the base population background from which the selected population was derived, (2) maintenance of the laboratory stocks and (3) the rearing conditions used in the following experiments.

Briefly, the parental generation for all the crickets was originally derived from eggs collected in dishes of sterilized earth placed in the base population or stock cage. The selected lines and the base population comprised both long-winged and short-winged females, but they differed in the proportion of long-winged or macropterous females (Roff *et al.*, 1999). The lines selected for high fecundity had a low frequency of long-winged morphs in both replicates (<10% long-winged), whereas the lines selected for low fecundity had higher proportions of long-winged females (Roff *et al.*, 1999). We only used the lines selected for low fecundity in the following experiment due to the technical resources required to collect enough long-winged females in the high fecundity lines. We started the selected population using offspring of 25 females taken from each low selected line described in Roff *et al.* (1999). These females were chosen indiscriminately from the last generation of the selection experiment. When the first individuals reached the penultimate instar, we placed them in a separate aquarium and subsequently used a pair of dishes each containing sterilized earth to collect eggs from this first generation. We started each of four subsequent generations from eggs laid in dishes containing sterilized earth. Each dish was left in the population cage for a week. Samples for the population study were initiated by placing dishes in the base and selected population cages at the same time.

We raised crickets in 4-litre buckets at 28°C under a 16:8 light:dark regime ('standard conditions' in Zera *et al.*, 1997) where they were provided with water and rabbit chow *ad libitum*. Fifty (early instar) individuals were placed in each 4-litre bucket. The base and selected population buckets were haphazardly mixed in the environmental chambers. Two samples (of 10 buckets) for each population were collected for the population comparison experiment. In each sample, after the first individuals reached the penultimate instar, the buckets were examined daily for females that had eclosed to adults. On the day they eclosed (designated day 1), females were placed in 1-litre plastic cages containing a water vial and excess rabbit chow. They were not provided with a loose damp substrate for oviposition, so they retained their eggs. Each newly eclosed individual was assigned a day for its preservation (days 1–7 after eclosion), in sequence, so that individuals from the same bucket or those which eclosed on the same day would not all be assigned the same preservation day.

On the date each female was preserved, its wing morph was first scored following Roff (1986) and then we collected 1 μ l of haemolymph from the severed tip of each female's cercus using a microcapillary pipette. Haemolymph acylglycerol (triglycerides and their derivatives) concentrations were determined on the same day using the assay described below. Females were preserved in Bouins fluid immediately after haemolymph sampling. After at least 2 weeks in the preservative, each female's head width was measured as an index of its body size, and then the ovaries and DLM were extracted and weighed.

Triglyceride assay. Zera and Mole (1994) extracted lipids from freeze-dried whole bodies of *Gryllus firmus* and argued that the difference in lipid concentrations between wing morphs was due to triglycerides, because long-winged females had higher average triglycerides than short-winged females. Because we needed to measure the weights of the DLM and ovaries of each female, we used a non-destructive method for sampling individual triglyceride concentrations. We used a Stanbio triglycerides kit (2001), which provided a peroxidase-coupled method for colorimetric determination of serum triglycerides. Triglycerides are insoluble storage products that are transported by the haemolymph largely as diacylglycerols (Downer, 1985). In the locust, for example, increased metabolic activity related to flight is accompanied by increasing diacylglycerol concentrations in the haemolymph (Beenakkers *et al.*, 1985). Haemolymph is both a transport organ and a storage organ for insect flight muscle fuels (Beenakkers *et al.*, 1985). The kit uses a non-specific lipase which hydrolyses triacylglycerols, diacylglycerols and monoacylglycerols (acylglycerols). If the biosynthesis and use of triglycerides and their derivatives (hereafter acylglycerols) are directly related to flight capability, then their concentrations should be highest in long-winged females with intact or non-histolysed DLM. One microlitre of cricket haemolymph was mixed with 1 ml of Stanbio reagent; the mixture was then incubated at room temperature for more than 10 min. The absorbance was read at 500 nm and each absorbance value was converted to $\text{mmol} \cdot \text{l}^{-1}$ triglycerides (acylglycerols).

Population comparisons: Statistical analyses

Wing morph proportion. We estimated average proportions of long-winged females in the population by counting the proportion of such females in each 4-litre bucket and then calculating the average (proportion) in the 20 sample buckets. Ovary weights, flight muscle weights and triglyceride concentrations all appear to change in the first week in *Gryllus firmus* (Zera *et al.*, 1994), so we collected a similar proportion of long-winged females from both populations on days 1–7 after the last eclosion.

Histolysis rates. Differences in histolysis rates could explain population differences in flight capability related characters and early reproduction (Fairbairn and Roff, 1990). To test this hypothesis, we divided the population into three groups: histolysed, intermediate and intact DLM. Histolysed DLM was defined as DLM from long-winged females with a smaller weight than DLM of day 1 short-winged females. Intact DLM were equal to or larger than the average size of DLM from long-winged females having no evidence of histolysis (category 5; Stirling *et al.*, 1999). We used a contingency analysis to test whether the proportion of long-winged females with histolysed DLM differed between populations. The test statistic was a χ^2 value, which was calculated as twice the negative log likelihood ratio.

Correlated selection. Based on the work of Zera *et al.* (1994, 1998), we expected (averaged over first week) long-winged females to have heavier DLM, higher serum acylglycerol concentrations and lower ovary weights than short-winged females. The microevolutionary trade-off hypothesis predicts selection for lower number of eggs laid will cause directional differences between base and selected populations in ovary weight (negative), DLM weight (positive) and acylglycerol concentration (positive). To test for significance of directional differences between wing morphs and between populations, a two-way analysis of variance

(ANOVA) was run with ovary weights, DLM weights and acylglycerol concentrations as dependent variables, and wing (long, short) and population (base, selected) as factors. A significant interaction between wing morph and population suggests short- and long-winged morphs did not have similar responses to selection.

In the absence of heritability estimates and information on the extent of inbreeding, Henderson (1997) suggested three decision rules can be used to test whether characters that are not under selection are genetically correlated with characters that have been selected. We used these decision rules and the direction of the difference between the base and selected populations (ovary weight = positive, flight muscle and triglyceride = negative) to test whether number of eggs laid, ovary weight, proportion long-winged, flight muscle size and triglyceride concentration are genetically integrated in a trade-off. Henderson (1997) argues that the standardized character difference ($\bar{Y}_{\text{selected}} - \bar{Y}_{\text{base}}/s_{\text{phenotype}}$, where s is standard deviation) can be used with simple decision rules to test whether a non-selected character (DLM weight, acylglycerol concentration, ovary weight) has a genetic relationship with the character under selection (number of eggs laid). He concluded that, if the standardized difference is less than 1, then the difference does not provide evidence of a genetic relationship. If it is between 1 and 2.2, then other supporting data should be obtained. In this case, other supporting data include: (1) the sign (configuration) of the change in wing morph, triglyceride concentration and DLM weight that are thought to have a functional (trade-off) relationship with ovary mass in long-winged females (Henderson, 1989); (2) the changes in these characters in short-winged females, since these characters are genetically correlated in the population even though these females are not flight capable. Finally, if the standardized difference is greater than 2.2, then there is most likely a genetic correlation between the two characters.

Phenotypic trade-offs within wing morphs: Statistical analysis

A physiological trade-off implies individual DLM size and acylglycerol concentrations should have a negative phenotypic relationship with early reproduction. This relationship is expected to differ between wing morphs, but it is not known how it changes in the first week after eclosion and whether it will be affected by selection.

We used an analysis of covariance (ANCOVA) model to test whether ovary weights of each wing morph are phenotypically related to DLM size or acylglycerol concentration. Either DLM size or acylglycerol concentration was the concomitant variable in a model that had population (base, selected) and day of the week as factors. Day of the week was a categorical character with seven levels. If the relationship between DLM weight or acylglycerol concentration is constant between days or populations (i.e. non-significant interactions), then the strength of the phenotypic relationship between ovary weight and DLM size (or acylglycerol concentration) can be tested using the significance of the overall slope coefficient.

When long-winged female *Gryllus firmus* eclose to adults, their flight muscle weights are large and the ovary weights of both wing morphs are relatively small. These characters subsequently change in size (Zera and Mole, 1994). Thus we would expect a statistical interaction between day and DLM weight (day-to-day changes in slope of the relationship between DLM weight and ovary weight). Zera *et al.* (1994) reported that triglyceride concentrations of long-winged females were higher than those of short-winged females overall

and that those of the long-winged morph changed during the first week after eclosion to an adult. This indicates there may also be an interaction between acylglycerol concentration and day of the week, if maintaining a higher acylglycerol concentration is related to a smaller increase in ovary weights. Most importantly, if there is a physiological trade-off between either DLM weight or acylglycerol concentration and ovary weight, we would expect a statistically significant negative relationship between them during the first week after eclosion.

RESULTS

DLM character correlation in the base population

Quantitative variation in DLM weight was strongly related to colour differences in long-winged females and histolysis-appearance categories in both wing morphs. The histolysis-appearance category, colour, volume and wet weight of DLM were all highly correlated in the base population (Table 1). One-way analyses of variance indicated average muscle weight differed significantly among both colour ($F_{4,33} = 19.0$, $P < 0.0001$) and appearance ($F_{4,33} = 52.6$, $P < 0.0001$) categories in long-winged females (Fig. 1). A *post-hoc* Tukey-Kramer analysis indicated that, in each category except category 5, the histolysis-appearance category mean weight of long-winged females did not differ significantly from the mean weight of colour categories with the same rank (Fig. 1). In other words, lighter coloured DLM appeared more histolysed. The histolysis measure was highly correlated with weight, volume and colour in all long-winged females (Table 1) and in long-winged females that had scores in the first three (1, 2, 3) histolysis-appearance categories (histolysis: weight, $r = 0.64$, $P < 0.001$; volume, $r = 0.42$, $P < 0.05$; colour, $r = 0.53$, $P < 0.01$, $n = 24$). Neither volume nor colour were correlated with DLM weight in these long-winged females, indicating DLM was being histolysed (Table 1). Short-winged females had lighter DLM, all of which were in categories 1–3. Nonetheless, a one-way ANOVA with DLM weight as the dependent variable and histolysis appearance as the factor indicated that the average DLM weight of short-winged females differed significantly among these three categories ($F_{2,20} = 5.67$, $P = 0.01$). DLM colour was not correlated with any of the other status measures in short-winged females (Table 1), consistent with the observations of Zera *et al.* (1988) that there are no significant differences in thoracic flight muscle colour of short-winged *Gryllus firmus*. However, the DLM weight of short-winged females was highly correlated with volume and histolysis-appearance measurements (Table 1). Long-winged categories 1, 2 and 3 also comprise DLM that are light-coloured, appear partially to fully histolysed and largely weigh less than 0.007 g (Fig. 1).

Microscopic examination of DLM. The histolysis-appearance category ranks corresponded to differences in microscopic appearance of DLM in long-winged females. However, the DLM of some short-winged females visually categorized as partly histolysed appeared to be intact. Microscopic examination of the DLM tissue sections indicated there was evidence of histolysis after day 3 and that long-winged flight muscle classified as non-histolysed had different structural characteristics than partially histolysed and fully histolysed flight muscle. Up to day 3, DLM fibres in all long- and short-winged crickets were relatively long with symmetrical cross-sections and they were highly nucleated. All

Table 1. Pairwise Spearman rank correlations (r) and their significance between four status measures of dorsal longitudinal flight muscle^a for both long- and short-winged morphs,^b short-winged (SW) morphs only^c and long-winged (LW) morphs only^d

	Weight	Volume	Colour	Histolysis appearance
Whole population				
Weight		0.82***	0.68***	0.87***
Volume			0.69***	0.81***
Colour				0.73***
SW morphs only above, LW morphs only below				
Weight		0.60**	-0.02	0.60**
Volume	0.79***		0.08	0.62***
Colour	0.72***	0.63***		0.00
Histolysis	0.89***	0.78***	0.78***	

^a Each measure (weight, volume, colour, histolysis) is described in the Methods section.

^b Correlations for both LW and SW are above the diagonal in the upper matrix (all five histolysis-appearance categories, $n = 62$).

^c The correlations for SW morphs (only) are above the diagonal in the lower matrix ($n = 23$).

^d The correlations for LW morphs (only) are below the diagonal in the lower matrix ($n = 38$).

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

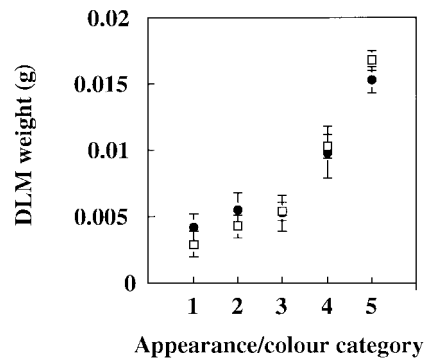


Fig. 1. Change in average DLM weight of long-winged females in histolysis-appearance categories (□) and colour categories (●). The appearance category is ranked from full histolysis (1) to intact or no evidence of histolysis (5). The colour categories are ranked from white (1) to dark pink (5). The error bars are ± 1 standard error. Some error bars are smaller than the symbol.

long-winged DLM in this group were scored as category 5 in both histolysis and colour categories, whereas short-winged flight muscle had lower category scores. In microscopic section, the DLM muscle fibres of long- and short-winged females appeared to be intact (up to day 3). The average number of fibres in short-winged females with DLM visually classified as partly histolysed, but apparently intact (in section), was significantly smaller than in long-winged females ($t_{10} = 2.6$, $P = 0.03$). The average fibre cross-section of short-winged females was also significantly smaller ($t_{10} = 4.51$, $P = 0.003$). Up to day 3,

short-winged females had average cross-section diameters (average number of fibres $\times$ average fibre cross-section) that were less than one-third that of long-winged females. After day 3, the DLM of long-winged females scored as partially histolysed differed in size and comprised fibres that varied from long but slightly shrivelled in cross-section to relatively short without intact nuclei. Both long- and short-winged flight muscle scored as fully histolysed were small in cross-section with fibres that were short, relatively narrow and empty, or were too highly stained to reveal any internal structure.

Population and sample descriptions

The proportion of long-winged females in the selected population was $44.8 \pm 6.4\%$ (mean $\pm$ standard error) and in the base population it was $22.7 \pm 3.6\%$. We tried to collect the same proportion of long-winged females in both populations, so that population comparisons would not include effects due to substantial differences in wing morph frequency for reasons discussed by Roff *et al.* (1999). The overall proportions of long-winged females were approximately equal in both population samples (selected sample: 44.2%, $n = 106$; base sample: 44.6%, $n = 87$).

Population comparisons

A factorial two-way ANOVA with head width as the dependent variable indicated that the interaction between population (base, selected) and wing morph (long, short) was not significant ($P > 0.05$), so we dropped the interaction term from the model and the analysis was re-run testing only the main factors. There was no significant difference in head width between the selected and base populations ($F_{1,1} = 0.32$, $P = 0.57$) or between long- and short-winged morphs ($F_{1,1} = 0.07$, $P = 0.93$). Consequently, we did not adjust ovary weights for head width, a surrogate measure of body size.

DLM histolysis and size differences. Long-winged females in both populations had DLM that weighed more than 0.006 g during the first 2 days after eclosion. This indicates that, later in the week, DLM lighter than 0.006 g (small DLM) is histolysed. In the base population, 32.2% ($n = 28$) long-winged females had small DLM, whereas in the selected population, only 22.5% ($n = 24$) had small DLM overall. However, the two populations did not differ significantly when the populations were divided into two groups that reflected the proportions with small and probably histolysed DLM (< 0.006 g) or larger DLM ($\chi^2 = 2.20$, $P = 0.14$). Even though a proportion of long-winged females appeared to be histolysing their DLM, the average daily DLM weight of long-winged females did not change significantly from days 3 to 7 in either population (base: $r^2 = 0.17$, $F_{1,65} = 1.17$, $P = 0.29$; selected: $r^2 = 0.03$, $F_{1,71} = 2.2$, $P = 0.14$).

If the DLM of short-winged females is also being histolysed, then there might be a concomitant decrease in DLM weight (from days 3 to 7). Overall, there were significant negative relationships in both the base ($F_{1,69} = 6.5$, $P = 0.01$; DLM weight = $0.0039 - 0.0003 \times \text{day}$) and selected populations ($F_{1,89} = 6.8$, $P = 0.01$; DLM weight = $0.0059 - 0.0004 \times \text{day}$). Sequential ANCOVA analysis revealed that the slopes were not significantly different ($R^2 = 0.17$, $F_{1,158} = 0.89$, $P = 0.35$), providing no evidence that histolysis rates in short-winged females differed between the two populations. However, the intercepts were significantly different ($F_{1,159} = 18.5$, $P < 0.0001$), indicating the short-winged females selected initially had larger DLM.

Correlated costs effect. The interactions between population and wing morph were not significant in each of the three ANOVA tests of overall differences (Table 2). The interaction terms were dropped from each model and the analysis was redone, but the significance of the main effects listed in Table 2 did not change. Overall, ovary weights were significantly smaller in the selected population, for both wing morphs (Fig. 2, Table 2). There were also significant differences between the base and selected populations in overall average

Table 2. Two-way ANOVA with ovary weight, DLM weight and acylglycerol concentration as the dependent variables

	Population ^a		Wing morph		Interaction ^b	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Ovary weight	12.3	0.0003	6.4	0.007	0.08	0.77
DLM weight	11.4	0.0004	342.8	< 0.0001	0.97	0.33
Acylglycerol concentration	9.2	0.001	21.1	< 0.0001	0.28	0.59

^a Population and wing morph are one-sided tests.

^b A two-sided null hypothesis of no difference.

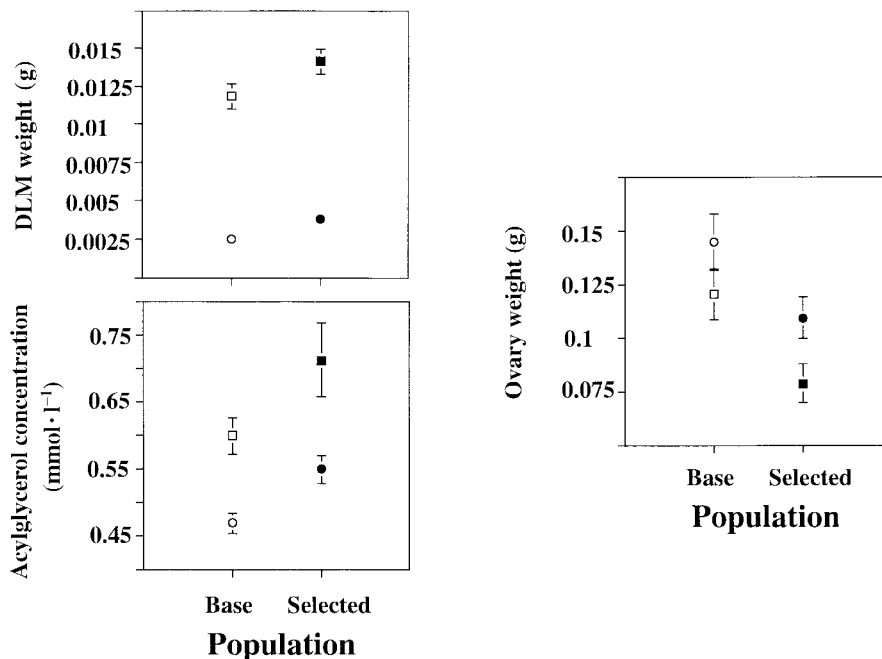


Fig. 2. Differences in three components of the flight fecundity trade-off (DLM weight, acylglycerol concentration and ovary weight) between the base (open symbol) and selected (closed symbol) populations. The symbols (squares = long-winged; circles = short-winged) represent overall averages and the error bars indicate ± 1 standard error. Some error bars are smaller than the symbol.

DLM weights and acylglycerol concentrations of both wing morphs (Table 2). The direction of these changes indicates that both DLM weight and acylglycerol concentration are negatively genetically correlated with early fecundity (Fig. 2). The differences in DLM weight, acylglycerol concentration and ovary weight were in the same direction for both wing morphs (Fig. 2).

In both morphs, the standardized differences of DLM weight and acylglycerol concentration between the base and selected populations were significant (Table 3). Standardized differences for the long-winged morph were larger than 1.8, but smaller than 2.2 (Table 3). The corresponding standardized differences in the short-winged morph were much larger than 2.2 (Table 3), providing strong corroborative evidence (Henderson, 1997) of negative genetic correlations between number of eggs laid on day 7 (character under selection), DLM weight and acylglycerol concentration.

Phenotypic relationships within wing morphs

Long-winged morph, DLM weight as the covariate. The full model accounted for a high proportion of variation in ovary weight ($F_{15,177} = 44.7$, $P < 0.0001$, $R^2 = 0.79$). There was no significant interaction between population and DLM weight ($F_{6,177} = 1.12$, $P = 0.29$), indicating relationships between DLM weight and ovary weight have similar slopes in the two populations. There was a significant interaction between day and DLM weight ($F_{6,177} = 6.71$, $P < 0.0001$). This indicates relationships between ovary weight and DLM weight differ through days 1–7 after adult eclosion. We pooled the data for both populations and estimated the common regression of ovary weight on DLM weight for each day after eclosion to adults. On day 1, when ovaries were small and did not contain fully formed eggs, the relationship between ovary weight and DLM weight was positive, providing evidence that there was no trade-off (Fig. 3). After day 2, the slopes of the relationship between DLM weight and ovary weight were negative (Fig. 3), consistent with the development of a phenotypic trade-off.

Long-winged morph, acylglycerol concentration as the covariate. The full model accounted for a high proportion of variation in ovary weight ($F_{15,174} = 19.7$, $P < 0.0001$, $R^2 = 0.63$). There was no significant interaction between population and acylglycerol concentration ($F_{6,174} = 0.6$, $P = 0.43$), indicating slopes of relationships in the base and selected populations to be similar. The interaction between day and acylglycerol concentration was significant ($F_{6,174} = 3.7$, $P = 0.0016$), indicating that there was a daily change in the relationship

Table 3. Standardized differences (t -statistic) within wing morphs, between base and selected populations

	Long-winged			Short-winged		
	t	P	n	t	P	n
Acylglycerol concentration	−1.81	0.04	190	−3.05	0.001	238
DLM weight	−2.03	0.02	193	−4.84	< 0.0001	242
Ovary weight	2.84	0.003	193	2.25	0.03	242

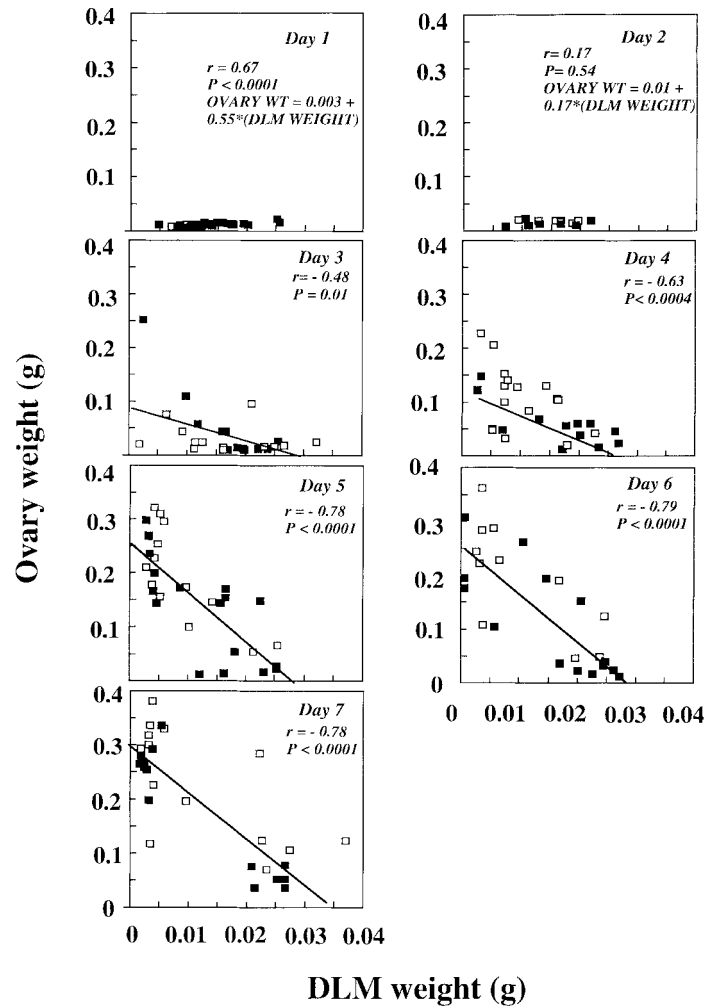


Fig. 3. Daily relationships between DLM weight and ovary weight of long-winged females from the base (□) and selected (■) populations. Each panel represents a different day after the last eclosion (1–7). In the first two panels, the regression equations are shown because the scale of the relationship is small. The lines on the other panels (days 3–7) represent the regression line.

between ovary weight and acylglycerol concentration. The data from both populations was pooled and on each day (days 1–7 after eclosion) ovary weight was regressed on acylglycerol concentration (Fig. 4). Similar to the relationship between DLM and ovary weight, the sign of the common regression became negative with ovary development, although the bivariate scatter indicates the relationship has a non-linear component after day 5 (Fig. 4).

Short-winged morph, DLM weight as the covariate. The full model accounted for a high proportion of the variation in short-winged ovary weight ($F_{15,226} = 47.43$, $R^2 = 0.78$,

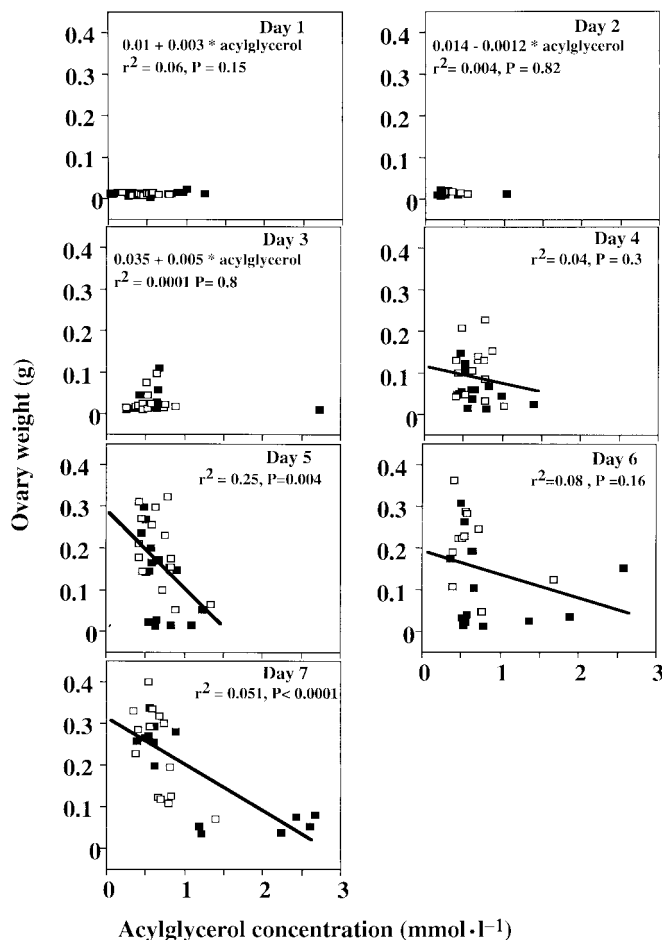


Fig. 4. Daily relationships between acylglycerol concentration and ovary weight of long-winged females from the base (□) and selected (■) populations. Each panel represents a different day after the last eclosion (1–7). In the first three panels, the regression equations are shown because the scale of the relationship is small. The lines on the other panels (days 4–7) represent the regression line.

$P < 0.0001$). The interaction between population and DLM weight was not significant ($F_{6,226} = 0.60$, $P = 0.44$), indicating relationships between DLM weight and ovary weight level have similar slopes. The interaction between day and DLM weight was also significant ($F_{6,226} = 2.95$, $P = 0.009$), indicating changes in the relationship between DLM weight and ovary weight of short-winged females in the first week. We pooled the base and selected data (common regression) and estimated the daily regression of ovary weight on DLM weight. Similar to long-winged morphs, the common relationship became negative with ovary development (Fig. 5). However, unlike the long-winged morphs, the range of short-winged DLM weights was small (approximately one-third of the long-winged range) and, by day 6, the DLM of most short-winged females was small, their ovary weights were large and the relationship between them was not significant (Fig. 5).

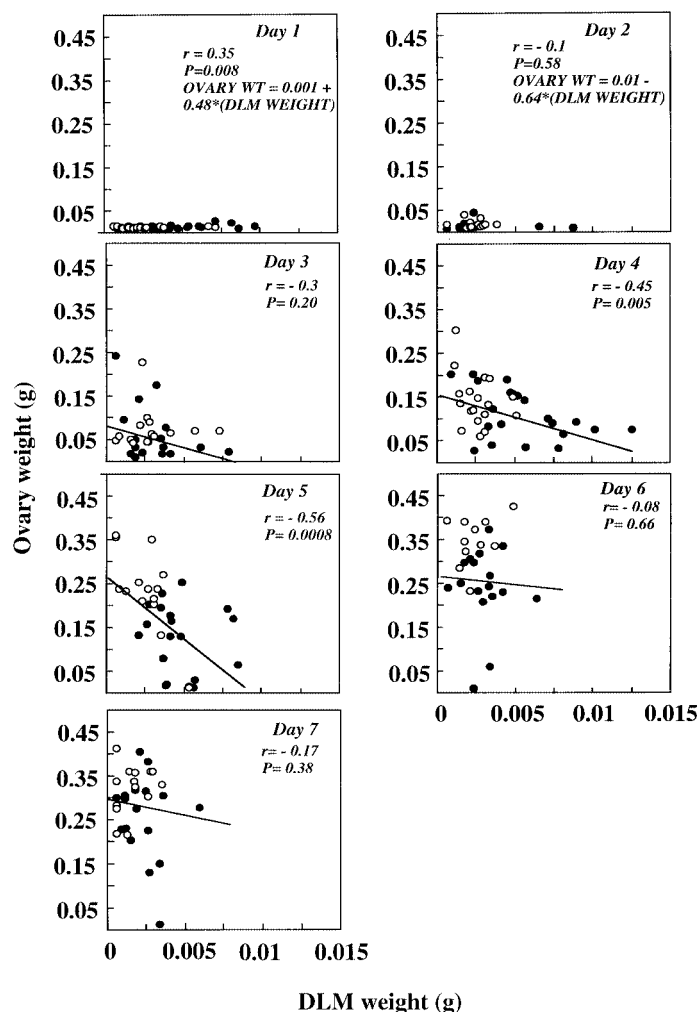


Fig. 5. Daily relationships between DLM weight and ovary weight of short-winged females from the base (○) and selected (●) populations. Each panel represents a different day after the last eclosion (1–7). In the first two panels, the regression equations are shown because the scale of the relationship is small. The lines on the other panels (days 3–7) represent the regression line.

Short-winged morph, acylglycerol concentration as the covariate. Neither the population $\times$ acylglycerol interaction ($F_{6,218} = 0.99$, $P = 0.31$) nor the day $\times$ acylglycerol interaction was significant ($F_{6,218} = 1.68$, $P = 0.13$). These results indicate that acylglycerol concentration in short-winged females does not differ significantly between populations or days in the first week after eclosion. We pooled all the data (across populations and day) but the regression of ovary weight on acylglycerol concentration was not significant ($r^2 = 0.008$, $F_{1,236} = 1.91$, $P = 0.17$). If the first 2 days are excluded, the relationship is weak ($r^2 = 0.045$) but significant and negative ($F_{1,160} = 7.61$, $P = 0.007$), although by day 5 it is not significant ($r^2 = 0.02$,

$F_{1,90} = 2.10$, $P = 0.15$). This suggests acylglycerol concentration may also have a weak and transient negative relationship with ovary weight in short-winged females.

Reproductive costs of flight capability

Figure 1 indicates that, by two qualitative measures (colour, histolysis appearance), DLM of long-winged females that are heavier than 0.014 g are also intact in the base population. In the selected population, 51.9% ($n = 55$) of long-winged females were in this category, whereas in the base population only 34.5% ($n = 30$) of long-winged females had DLM heavier than 0.014 g. A contingency analysis indicated that, when the populations were divided into two categories (equal to or greater than 0.014 g, less than 0.014 g), the overall proportions of long-winged females in these categories differed significantly between the populations ($\chi^2 = 5.9$, $P = 0.01$). A one-way ANOVA indicated there were significant differences between the acylglycerol concentrations of long-winged females with large DLM (greater than 0.014 g) and other females (long-winged with smaller DLM and short-winged: $F_{2,425} = 27.14$, $P < 0.0001$). A *post-hoc* Tukey-Kramer analysis showed that the average acylglycerol concentration of long-winged females with large DLM (0.81 ± 0.034 mmol $\cdot$ l $^{-1}$; mean $\pm$ standard error) was significantly larger than that of long-winged females with smaller DLM (0.54 ± 0.034 mmol $\cdot$ l $^{-1}$) and that of short-winged females (0.51 ± 0.021 mmol $\cdot$ l $^{-1}$). Long-winged females with large DLM also had significantly smaller ovary weights (0.075 ± 0.0137 g, $n = 49$) than both short-winged females (0.206 ± 0.008 g, $n = 132$) and long-winged females with smaller DLM (0.209 ± 0.0118 g, $n = 67$). This result indicates the common, early reproductive cost of flight capability is a 64% reduction in development of mature eggs in the ovary. However, a *t*-test ($t_{47} = 2.5$, $P = 0.016$) indicated that selected long-winged females with large DLM had significantly smaller ovary weights on average (0.06 ± 0.01 g, $n = 33$) than females with large DLM in the base population (0.10 ± 0.014 g, $n = 16$), indicating the reproductive cost of flight capability had evolved from a 52% reduction in the base population to a 71% reduction of ovary weight of flight-capable long-winged morphs in the selected population. This estimate ignores the related reduction in short-winged fecundity (Fig. 2).

DISCUSSION

In this study, we tested the hypothesis that wing muscle size and triglyceride concentration are part of a functional relationship that mediates the expression of genetic relationships governing a life-history trade-off between wing morph and early fecundity. There is not a large material investment in longer wings and the wings are usually fully formed by the end of the first day after the final eclosion, so wing length itself probably has little, if any, reproductive cost. At the same time, wing morph has long been considered to indicate a syndrome of characters (i.e. flight capability) associated with each morph (Dingle, 1985; Roff, 1986). However, Fairbairn (1994) has argued that this classification ignores within-wing morph variability of other characters in the trade-off. We used this within-morph variability to test whether putative functional relationships between flight muscle size or triglyceride concentration and early fecundity are negative in female sand crickets. Both wing muscle size (indicated by DLM size) and triglyceride concentration (indicated by acylglycerol concentration) had negative phenotypic correlations with ovary weight, consistent with this trade-off hypothesis. We also found that DLM weight or size (Table 1),

triglyceride concentration and ovary weight differed between a population selected for low number of eggs laid in the first week and the base population, in directions predicted by the trade-off model. These results are consistent with the interpretation that negative genetic correlations between wing morph frequency and early fecundity (Roff *et al.*, 1997, 1999) reflect constraints on early reproduction, imposed by the genetic architecture of a trade-off through functional relationships (early fecundity with flight capability-related characters), rather than being an arbitrary assumption of a trade-off model (Tuomi *et al.*, 1983; Charlesworth, 1990).

Phenotypic relationships

Before performing the experiment reported here, differences in wing muscle status were scored on several qualitative scales, but our data suggest all these measures to be highly correlated with DLM weight. This result indicates that variation in DLM weight, whether it is due to histolysis or not, can be treated quantitatively rather than as categorical differences in histolysis condition appearance (Roff, 1989) or colour (Zera *et al.*, 1998). Quantitative variation in DLM weight had a strong negative relationship with ovary weight within both wing morphs, although this relationship developed a few days after females eclosed to adults under standard conditions. A detailed examination of the phenology of the DLM weight–ovary weight relationship shows phenotypic relationships are negative in both wing morphs, as long as ovaries are developing and DLM is not completely histolysed. These results are consistent with predictions that DLM status in long-winged females is a functionally integrated reproductive cost. It is also a novel finding that the relatively small flight muscle size of short-winged females has a transient negative phenotypic relationship with ovary weight. Acylglycerol concentration – and, by implication, triglyceride concentration – had negative phenotypic effects on ovary development (weight) in the long-winged morph, but this relationship was at best weak and transient in short-winged morphs. Furthermore, long-winged females that did not have large DLM and were presumably flight-incapable (Fairbairn and Roff, 1990), had acylglycerol concentrations and ovary weights that were not significantly different from short-winged females. At the same time, long-winged females with the largest DLM had the highest acylglycerol concentrations and the smallest ovary weights. The difference between wing morphs in terms of average concentrations of mobile, high-energy lipids supports the idea that flight-capable long-winged females have higher energetic demands due to the maintenance of larger flight muscles (Zera and Mole, 1994; Zera *et al.*, 1998). In these three groups – short-winged, long-winged with small DLM, long-winged with large DLM – the overall results were consistent with the expectation that DLM size and acylglycerol concentration mediate reproductive costs of flight capability. The acylglycerol concentrations of long-winged females with large (intact) DLM increased towards the end of the week (Fig. 4). This increase may represent the metabolic cost of putting energy into both the maintenance of flight capability and vitellogenesis. In summary, these results support the idea that DLM size (Roff, 1986) and the associated triglyceride concentrations (Mole and Zera, 1994) mediate, at least in part, the phenotypic trade-off between wing morph and early fecundity in crickets. Differences between the base and selected populations suggest these functional relationships had evolved, but these differences may be related to wing morph proportion, differences in histolysis rates or a genetic correlation underlying functional relationships mediating the phenotypic trade-off.

Wing morph proportion

The proportion of macropterous individuals differed between populations (selected = 45%, base = 23%). If the populations had been compared using these wing morph frequencies, the effect of wing morph proportion would have been low. Assuming long-winged morphs in the selected population have the average ovary weight estimated for the base population, the difference in the proportion macropterous (22%) would change ovary weight by 3.9% (using eqns 6 and 7 in Roff *et al.*, 1999). This percentage difference in ovary weight was similar to the percentage difference in the number of eggs laid due to a correlated increase in the proportion of long-winged morphs in the fourth selected generation (3–4%; Roff *et al.*, 1999).

Histolysis, ovary development and DLM size

Roff *et al.* (1999) note that fecundity of the long-winged morph could be radically changed through a genetically correlated change in the schedule of flight muscle histolysis (see Fairbairn and Roff, 1990; Roff, 1994). Based on the proportion of small (less than 0.006 g) DLM we could not reject the (null) hypothesis that histolysis rates were similar in both populations. These results also indicate the difference in ovary weights between the base and selected population cannot be attributed to differences in nutrients generated by different histolysis rates, suggesting this mechanism (Tanaka, 1993; Gomi *et al.*, 1995) does not regulate the trade-off. Nonetheless, the synchrony between ovary growth and DLM histolysis is remarkable during the first week in both populations.

At the same time, the pattern of histolysis differed between wing morphs. There was a progressive reduction in the average values of DLM weight in short-winged females between days 4 and 7, whereas the larger average DLM weight of long-winged females did not change significantly. Consequently, almost all the short-winged females had very small DLM on day 7 (less than 0.005 g), suggesting their DLM was histolysed (Fig. 5). Conversely, Fig. 3 indicates that, by day 7, long-winged females either had DLM that was large, pigmented and intact (heavy) or small, light in colour and histolysed (light). The proportion of long-winged females with DLM heavier than 0.014 g early in the week (days 1 and 2) was not significantly different from the proportion of heavy DLM late in the week (days 6 and 7) in both the base (early 35.0%, $n = 7$; late 34.6%, $n = 9$; $\chi^2 = 0.98$) and selected populations (early 36.4%, $n = 12$; late 51.7%, $n = 15$; $\chi^2 = 0.22$). This implies there is a size threshold below which DLM is histolysed by day 7 (regardless of the wing morph), and explains why average DLM weight did not decline significantly after day 3 in long-winged morphs.

Are flight capability and early fecundity genetically integrated?

If neither wing morph frequency nor histolysis can explain the differences in ovary weights between the base and selected populations, is it plausible that negatively genetically correlated flight capability-related characters mediate the trade-off between wing morph and early fecundity? Roff *et al.* (1999) reported a significant response to selection for a component of early fecundity (lower total number of eggs laid in the first week after the last eclosion) and a significant correlated increase in long-winged female proportions in the selected *Gryllus firmus* population. In this study, we confirmed that the increase in the

long-winged proportion is a stable response; that is, the correlated increase in this flight capability-related character cannot be attributed to transient linkage disequilibrium caused by repeated, strong directional selection. This suggests that increases in DLM weight or triglyceride concentrations in the selected population are also stable responses to selection for lower number of eggs laid. Selected long-winged females with large DLM have ovary weights that are only 29% as large as the common estimate for short-winged females (or long-winged females with small or intermediate DLM). Base population long-winged morphs with large DLM have ovary weights that are 48% as large. This result, together with the decrease in the number of eggs laid (Roff *et al.*, 1999), indicate reproductive differences (costs) covary with flight capability-related characters (genetic integration; Cheverud, 1996). Two approaches were suggested by Henderson (1989, 1997) to determine whether such character differences between unreplicated lines are spurious relationships caused by drift, or are due to genetic correlations.

First, Henderson (1989) suggested examining the direction of change in several characters predicted to have functional relationships with the selected character (number of eggs laid). We have argued above that DLM size and triglyceride concentrations have functional relationships with the level of early fecundity expressed. The direction of change in acylglycerol concentrations (triglycerides) and DLM weight from the base to the selected population are both consistent with the prediction that they are negatively genetically correlated with early fecundity, which decreases.

Second, Henderson (1997) suggested using the size of the effect on the putative correlated character, since the effects of drift are generally smaller than that of correlated selection. Based on both analytic arguments and numeric analyses, Henderson (1997) argued that, if the selected line is not highly inbred, then by using the standardized phenotypic difference between lines, we can employ simple decision criteria described in the Methods section to test whether characters are genetically correlated or not. We applied Henderson's (1997) decision rules to differences within morphs and between the selected population and the base population from which it was derived. Our results provide evidence for substantive genetic relationships between DLM weight or acylglycerol concentration and the selected character (the number of eggs laid).

The direction of differences between base and selected populations in both wing morphs, the overall changes in flight capability-related characters (or reproductive costs) in both wing morphs and the demonstration of a transient functional trade-off in short-winged females each provide strong supporting evidence for the existence of negative genetic correlations (between early fecundity and DLM size or triglyceride concentration) underlying functional relationships, which regulate the wing morph–early fecundity life-history trade-off in this *Gryllus firmus* population. To test the generality of this life-history trade-off model or the stability of its functional relationships, we need to estimate genetic parameters for characters involved in the trade-off. These estimates can be used to predict how life-history relationships might change across other populations with different wing morph frequencies, different cricket species or other wing dimorphic insect genera. However, genetic correlations reflect both genetic and functional relationships (Houle, 1991; Stearns *et al.*, 1991). Our results indicate estimates of genetic parameters could depend on the initial distribution of DLM size and the timing of DLM histolysis. Although histolysis could govern the strength or significance of the flight capability–early fecundity life-history trade-off, it does not appear to establish or regulate differences in functional relationships of sand crickets, *Gryllus firmus*.

ACKNOWLEDGEMENTS

Many thanks to Martin Cayer, without whose technical assistance we would not have been able to perform this experiment, Dr R. Levine for advice on histological technique and Dr N. Price for access to a multibeam spectrophotometer. Drs R. Latta, P. Crnokrak and D. Réale reviewed earlier versions of the manuscript. Their comments are much appreciated. This work was supported by a NSERC collaborative grant to Daphne J. Fairbairn and Derek A. Roff.

REFERENCES

- Beenackers, A.M., Van der Horst, D.J. and Van Marrewijk, W.J.A. 1985. Biochemical processes directed to flight muscle metabolism. In *Comprehensive Insect Physiology and Pharmacology*, Vol. 10 (G.A. Kerkut and L.I. Gilbert, eds), pp. 451–486. Oxford: Pergamon Press.
- Charlesworth, B. 1990. Optimization models, quantitative genetics, and mutation. *Evolution*, **44**: 520–538.
- Cheverud, J.M. 1996. Developmental integration and the evolution of pleiotropy. *Am. Zool.*, **36**: 44–50.
- Dingle, H. 1985. Migration. In *Comprehensive Insect Physiology and Pharmacology*, Vol. 9 (G.A. Kerkut and L.I. Gilbert, eds), pp. 375–416. Oxford: Pergamon Press.
- Downer, R.G.H. 1985. Lipid metabolism. In *Comprehensive Insect Physiology and Pharmacology*, Vol. 10 (G.A. Kerkut and L.I. Gilbert, eds), pp. 77–113. Oxford: Pergamon Press.
- Fairbairn, D.J. 1994. Wing dimorphism and the migratory syndrome: Correlated traits for migratory tendency in wing dimorphic insects. *Res. Pop. Ecol.*, **36**: 157–163.
- Fairbairn, D.J. and Roff, D.A. 1990. Genetic correlations among traits determining migratory tendency in the sand cricket, *Gryllus firmus*. *Evolution*, **44**: 1787–1795.
- Gomi, T., Okuda, T. and Tanaka, S. 1995. Protein synthesis and degradation in the flight muscles of adult crickets (*Gryllus bimaculatus*). *J. Exp. Biol.*, **198**: 1071–1077.
- Gustafsson, L., Nordling, D., Anderson, M.S., Sheldon, B.C. and Qvarnstrom, A. 1994. Infectious diseases, reproductive effort and the cost of reproduction in birds. *Phil. Trans. R. Soc. Lond.*, **346**: 323–331.
- Henderson, N.D. 1989. Interpreting studies that compare high- and low-selected lines on new characters. *Behav. Genet.*, **19**: 473–502.
- Henderson, N.D. 1997. Spurious associations in unreplicated selected lines. *Behav. Genet.*, **27**: 145–154.
- Houle, D. 1991. Genetic covariance of fitness correlates: What genetic correlations are made of and why it matters. *Evolution*, **45**: 630–648.
- Johnson, C.G. 1969. *The Migration and Dispersal of Insects by Flight*. London: Methuen.
- Mole, S. and Zera, A.J. 1993. Differential allocation of resources underlies the dispersal–reproduction trade-off in the wing-dimorphic cricket, *Gryllus rubens*. *Oecologia*, **93**: 121–127.
- Mole, S. and Zera, A.J. 1994. Differential resource consumption obviates a potential flight–fecundity trade-off in the sand cricket (*Gryllus firmus*). *Funct. Ecol.*, **8**: 573–580.
- Partridge, L. 1992. Measuring reproductive costs. *Trends Ecol. Evol.*, **7**: 99–100.
- Reznick, D. 1992. Measuring the costs of reproduction. *Trends Ecol. Evol.*, **7**: 42–46.
- Riska, B. 1989. Composite traits, selection response and evolution. *Evolution*, **43**: 1172–1191.
- Roff, D.A. 1984. The cost of being able to fly: A study of wing polymorphism in two species of crickets. *Oecologia*, **63**: 30–37.
- Roff, D.A. 1986. The evolution of wing dimorphism in insects. *Evolution*, **40**: 1009–1020.
- Roff, D.A. 1989. Exaptation and the evolution of dealation in insects. *J. Evol. Biol.*, **2**: 109–123.
- Roff, D.A. 1994. Evidence that the magnitude of a trade-off in a dichotomous trait is frequency dependent. *Evolution*, **48**: 1650–1656.
- Roff, D.A. 1997. *Evolutionary Quantitative Genetics*. New York: Chapman & Hall.

- Roff, D.A. and Bradford, M.A. 1996. Quantitative genetics of the trade-off between fecundity and wing dimorphism in the cricket *Allonemobius socius*. *Heredity*, **76**: 178–185.
- Roff, D.A. and Fairbairn, D.J. 1991. Wing dimorphisms and the evolution of migratory polymorphisms among the insecta. *Am. Zool.*, **31**: 243–251.
- Roff, D.A., Stirling, G. and Fairbairn, D.J. 1997. The evolution of threshold traits: A quantitative genetic analysis of the physiological and life history consequences of wing dimorphism in the sand cricket. *Evolution*, **51**: 1910–1919.
- Roff, D.A., Tucker, J., Stirling, G. and Fairbairn, D.J. 1999. The evolution of threshold traits: Effects of selection on fecundity and correlated response in wing dimorphism in the sand cricket. *J. Evol. Biol.*, **12**: 535–546.
- Sinervo, B. and Basolo, A.L. 1996. Testing adaptation using phenotypic manipulations. In *Adaptation* (M.R. Rose and G.V. Lauder, eds), pp. 149–185. San Diego, CA: Academic Press.
- Stearns, S.C. 1992. *Evolution of Life Histories*. New York: Oxford University Press.
- Stearns, S.C., de Jong, G. and Newman, B. 1991. The effects of phenotypic plasticity on genetic correlations. *Trends Evol. Ecol.*, **6**: 122–126.
- Stirling, D.G., Fairbairn, D.J. and Roff, D. A. 1999. Four characters in a trade-off: Dissecting their phenotypic and genetic relations. *Oecologia*, **120**: 492–498.
- Tanaka, S. 1993. Allocation of resources to egg production and flight muscle development in a wing dimorphic cricket, *Modicogryllus confirmatus*. *J. Insect Physiol.*, **39**: 493–498.
- Tuomi, J., Hakala, T. and Haukioja, E. 1983. Alternative concepts of reproductive effort, costs of reproduction and selection in life-history evolution. *Am. Zool.*, **23**: 25–34.
- Wheeler, D. 1996. The role of nourishment in oogenesis. *Ann. Rev. Entomol.*, **41**: 407–431.
- Zera, A.J. and Denno, R.F. 1997. Physiology and ecology of dispersal polymorphism in insects. *Ann. Rev. Ecol. Syst.*, **42**: 207–230.
- Zera, A.J. and Mole, S. 1994. The physiological costs of flight capability in wing-dimorphic crickets. *Res. Pop. Ecol.*, **32**: 151–156.
- Zera, A.J., Mole, S. and Rokke, K. 1994. Lipid, carbohydrate and nitrogen content of long and short winged *Gryllus firmus*: Implications for the physiological cost of flight capability. *J. Insect Physiol.*, **40**: 1037–1044.
- Zera, A.J., Sall, J. and Grudzinski, K. 1997. Flight muscle polymorphism in the cricket *Gryllus firmus*: Muscle characteristics and their influence on the evolution of flightlessness. *Physiol. Zool.*, **70**: 519–525.
- Zera, A.J., Potts, J. and Kobus, K. 1998. The physiology of life-history trade-offs: Experimental analysis of a hormonally induced life-history trade-off in *Gryllus assimilis*. *Am. Nat.*, **152**: 7–23.

