The evolution of threshold traits: effects of selection on fecundity and correlated response in wing dimorphism in the sand cricket

D. A. ROFF,* J. TUCKER,† G. STIRLING* & D. J. FAIRBAIRN†

*Department of Biology, McGill University, 1205 Dr Penfield Ave., Montreal, Quebec, Canada, H3A 1B1
†Department of Biology, Concordia University, 1455 de Maisonneuve Blvd., West, Montreal, Quebec, Canada H3G 1M8

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Abstract

The quantitative genetic basis of traits can be determined using a pedigree analysis or a selection experiment. Each approach is valuable and the combined data can contribute more than either method alone. Analysis using both sib analysis and selection is particularly essential when there are likely to be nonlinearities in the functional relationships among traits. A class of traits for which this occurs is that of threshold traits, which are characterized by a dichotomous phenotype that is determined by a threshold of sensitivity and a continuously distributed underlying trait called the liability. In this case, traits that are correlated with the liability may show a nonlinear relationship due to the dichotomy of expression at the phenotypic level. For example, in wing dimorphic insects fecundity of the macropterous (long-winged) females appears in part to be determined by the allocation of resources to the flight muscles, which are almost invariably small or absent in the micropterous (short-winged, flightless) females. Pedigree analysis of the cricket *Gryllus firmus* has shown that wing morph, fecundity and the trade-off between the two have additive genetic (co)variance. It has also been shown that selection on proportion macroptery produced an asymmetric correlated response of fecundity. The present paper details the results of direct selection on fecundity and the correlated response in proportion macroptery. Selection for increased fecundity resulted in increased fecundity within both wing morphs and a correlated decrease in proportion macroptery. Similarly, selection for decreased fecundity resulted in a decrease within morphs and a correlated increase in the proportion of macropterous females. This provides additional evidence that the trade-off between fecundity and wing morphology has a genetic basis and will thus modulate the evolution of the two traits.

Introduction

Directional selection produces a change in the population mean of a phenotype, but this shift will only be translated into a phenotypic difference between generations if the trait is heritable, in the sense that there is additive genetic variance in the trait. Thus any study of evolutionary change must concern itself both with the factors causing phenotypic change and the genetic architecture of the traits under investigation. In the case of a single trait the relevant genetic parameter is the narrow sense heritability, defined as the ratio of the additive genetic variance to the phenotypic variance (Falconer, 1989). Heritability can be estimated by pedigree analysis (e.g. full-sibs, half-sibs) or by response to selection, the latter estimate frequently being called the realized heritability. Estimation of the heritability from pedigree analysis is the more generally adopted method, because it is typically both less labour-intensive and quicker, requiring only one or two generations of breeding.

A selection experiment is not simply an alternative method of estimating heritability but can provide insights into the genetic architecture of a trait that are not evident from the sib analysis. For example, according to the standard response equation (Response = Heritability ×

Correspondence: Dr Derek A. Roff, Department of Biology, McGill University, 1205 Dr Penfield Ave., Montreal, Quebec, Canada, H3A 1B1.
Tel: 514 398 6464; fax: 514 398 5069; e-mail: droff@bio1.lan.mcgill.ca

Selection differential) the response should be the same in both directions of selection. This is frequently not the case (see Table 4.2, Roff, 1997), and many reasons for asymmetry in response have been advanced (Roff, 1997, pp. 134–137).

A central feature of evolutionary theory, particularly that associated with the evolution of life history traits, is that trait evolution is a function of the interaction of several traits (Roff, 1992; Stearns, 1992). Selection favouring an increase in a particular trait may be opposed by a fitness trade-off between this trait and a second trait. As with directional selection on a single trait, for the trade-off to be evolutionarily important in determining the trajectory of trait values and the final equilibrium combination, the trade-off must be determined in part by additive genetic variance. This genetic determination is measured by the additive genetic correlation between the two traits. As a consequence of the genetic correlation, selection on one trait will produce a change in other traits with which it is correlated. As with the response to selection, the 'simple' correlated response equation predicts that the correlated response will be symmetric. But, as might be expected from the frequent occurrence of asymmetric direct responses to selection, the correlated response often depends upon the direction of direct selection (Roff, 1997, pp. 171–182). The genetic analysis of two traits thus involves both a pedigree analysis and two selection experiments: divergent selection (i.e. one 'up' line and one 'down' line) on trait X with measurement of the direct response of X and the correlated response of trait Y, and divergent selection on trait Y with measurement of the direct response of Y and the correlated response of trait X.

The above triad of experiments is particularly important if there are nonlinearities in the phenotypic expression of one or more of the traits under study. In such cases, even if there is continuity at the genetic level, the direct and correlated responses may not be symmetric. Threshold traits are quantitative genetic traits for which there is a clear nonlinearity at the phenotypic level. These traits are characterized by two phenotypically distinct morphs that are assumed to be genetically determined by a continuously distributed underlying trait called the liability (Falconer, 1989). Individuals which have liabilities above a particular threshold develop into one morph while individuals below the threshold develop into the alternative. Examples of threshold traits are widespread (reviewed in Roff, 1996) and include cyclomorphosis, paedomorphosis, dimorphism in morphology (e.g. weaponry, trophic structures, wings), in reproductive behaviour or life history (e.g. semelparity vs. iteroparity; diapause vs. direct development), sex determination in some insects and reptiles, twinning in some mammals, and responses to physiological challenges (e.g. disease resistance). The presence of two distinct morphs suggests that there are both costs and benefits associated with each morph. In this regard, wing dimorphism in insects has been particularly well studied and serves as a model for the evolution of threshold traits.

Wing dimorphism is found in many different taxa of insects and comprises a winged morph that is typically capable of flight, and a short-winged or wingless morph that cannot fly. The primary feature of the habitat of wing dimorphic insects (we here exclude sexual dimorphisms) is that they form a mosaic of patches that are spatially and temporally heterogeneous (den Boer, 1970, 1979; den Boer *et al.*, 1979; Dingle, 1985; Roff, 1990a; 1994a; Denno *et al.*, 1991). In such an environment, migration of at least part of the population is essential for long-term persistence (reviewed in Roff, 1994b). However, being capable of flight considerably reduces the fecundity of females (Roff, 1986; Roff & Fairbairn, 1991) and in males decreases the success of obtaining mates (Crnokrak & Roff, 1995; Fairbairn & Preziosi, 1996). A trade-off between the two traits, wing morphology and reproduction, is thus maintained by the balance between the benefits of present reproductive success and long-term persistence (Roff, 1994b). Similar trade-offs between the threshold trait and reproductive parameters have been observed in other types of threshold traits (Roff, 1996).

The trade-offs reported for wing dimorphism and other threshold traits have in almost all cases been documented only at the phenotypic level. As described above, it is minimally necessary to demonstrate that these trade-offs have a genetic basis, which means showing that the two traits in the trade-off are genetically correlated (Reznick, 1985). The heritability of threshold traits is typically large enough (>0.30; Roff, 1998a) to respond to modest selection intensity, and life history traits such as fecundity have heritabilities frequently between 0.15 and 0.30 (Mousseau & Roff, 1987; Roff & Mousseau, 1987; Weigensberg & Roff, 1996), suggesting that a genetic correlation between a threshold trait and a reproductive trait such as fecundity is plausible. Estimation by pedigree analysis of the genetic correlation between wing morphology and fecundity in the two cricket species *Allonemobius socius* (Roff & Bradford, 1996) and *Gryllus firmus* (Roff, 1994c; Roff *et al.*, 1997) has shown that the trade-off is genetically based and thus will potentially modulate the joint evolution of the two traits.

Directional selection on proportion macropterous (long-winged) in *G. firmus* produced a rapid direct response in accord with the heritability estimated from both full-sib (Roff, 1994c) and half-sib (Roff *et al.*, 1997) experiments. There was, however, asymmetry in the response, with selection for decreased macroptery producing a faster response than selection for increased macroptery (Roff, 1990b). Analysis of the correlated response in fecundity showed that the fecundity of the macropterous females in the lines selected for high proportion macroptery decreased relative to the macropterous females from the control line, and was in

reasonable accord with predictions from the correlated response equation (Roff, 1994c). However, the fecundity of the micropterous (short-winged) females in the lines selected for low proportion macroptery did not differ statistically from the fecundity of the micropterous females from the control lines. Roff (1994c) suggested that this asymmetrical response resulted from the presence of the large flight muscles in the macropterous females. The trade-off between fecundity and wing morphology is hypothesized to be due to competition for resources between the gonads and the flight muscles (Roff, 1984; Roff & Fairbairn, 1991; Zera & Denno, 1997; Zera *et al*., 1997): selection for increased macroptery changes the pattern of allocation and changes the early fecundity of macropterous females. Because they lack the flight muscles, micropterous females should not be affected by this shift in allocation and hence there should little correlated response (Roff, 1994c).

From the heritabilities and genetic correlation estimated from the pedigree analyses we predict that selection for increased fecundity in *G. firmus* will decrease the proportion of macropterous females, while selection for decreased fecundity will increase the proportion of macropterous females. However, the asymmetry of the response to selection on macroptery makes such predictions uncertain. If fecundity were itself dichotomous, with each morph having a characteristic value, a shift solely due to a change in morph frequency would occur (hereafter referred to as the 'dichotomy' hypothesis). Such a scenario is generally assumed in the analysis of threshold traits (Lively, 1986; Moran, 1992; Pfennig, 1992; Rowell & Cade, 1993; Roff, 1994b), although it has been shown to be theoretically unlikely (Fairbairn, 1986, 1994; Fairbairn & Roff, 1990). The appropriate genetic model is for a trait such as fecundity to be genetically correlated with the underlying continuously distributed trait (= liability; for a discussion of liability see Falconer, 1989, and for its extension to the correlation between two traits see Mercer & Hill, 1984), and as a consequence selection on the proportion macropterous will cause a shift in trait values within morphs (Fairbairn & Roff, 1990; Fairbairn, 1994). Such shifts in the macropterous morph were observed in fecundity (Roff, 1994c), and in rates of flight muscle histolysis and flight propensity (Fairbairn & Roff, 1990), providing evidence against the dichotomy hypothesis. On the other hand, the asymmetry of the fecundity response suggests that we must incorporate a nonlinearity into the model possibly due to a nonlinear relationship between muscle size, wing morph and fecundity. It can be shown (see Appendix) that such a model can produce an asymmetrical correlated response in fecundity when direct selection is applied to wing morph but a symmetrical correlated response in the liability when direct selection is applied to fecundity. Given this potential complexity it is essential to measure the correlated response of macroptery to selection on fecundity.

Materials and methods

Organism and experimental protocol

Gryllus firmus is a relatively large (≈0.7 g) wing-dimorphic cricket that typically inhabits sandy sites along the eastern seaboard of the United States (Alexander, 1968; Harrison, 1985). The individuals used in the present experiment were from a stock culture that originated from ≈20 males and 20 females collected in northern Florida in 1981. The stock culture is maintained with a standing adult population of several hundred individuals. To prevent diapause the temperature is maintained in excess of 25 °C. Nymphs and adults in both the stock and the selection experiments were fed Purina rabbit chow.

The selection protocol followed a standard mass selection design with two lines selected for increased fecundity, two lines selected for decreased fecundity and four control lines. The 'high fecundity' and 'low fecundity' selection experiments were initiated at different times and followed slightly different protocols, the most important difference being in the definition of fecundity in the two experiments. From previous experiments (Roff, 1994c; Roff *et al*., 1997) it has been shown that total fecundity, measured as eggs laid in the first week after eclosion plus the fully formed eggs in the ovaries, has a higher heritability and is more normally distributed than the number of eggs laid. We therefore selected on total fecundity in the following manner. Adult females were separated into individual cages, each with a mate, food, water and an oviposition dish, and the number of eggs laid in the first 7 days post eclosion measured. The females were then killed and the number of eggs in the ovaries counted. The eggs laid by the 25 females with the highest total fecundity were used to form the next generation, each female contributing the same number of offspring.

The above protocol could not be followed when selecting for reduced fecundity because in this case the required females typically did not lay any eggs in the first week after eclosion, and thus had to be retained for several weeks to obtain the required eggs. For the low fecundity lines we therefore selected on eggs laid in the first week, the 25 females laying the lowest number of eggs being used as parents of the next generation. Because of the different protocols the experiment is not strictly a bidirectional selection experiment: to avoid confusion we shall refer to the high fecundity line as 'high total fecundity line' and the low fecundity line as 'low eggs laid line'

To initiate the 'high total fecundity' experiment 25 adult females were chosen haphazardly from the stock culture and from each female 32 nymphs obtained. The 800 nymphs were distributed among 16 cages, each cage receiving two nymphs from each female (giving 50 nymphs per cage): this generation is designated as gener-

ation 1. Upon final eclosion, 100 first-generation females were selected at random and the 25 females with the highest fecundity were used to initiate two high total fecundity selection lines. Each female contributed 24 nymphs to each of the two selection lines, these lines being thereafter treated independently. In each subsequent generation the fecundities of 100 randomly selected females in each line were measured and the 25 females with the highest fecundities selected as parents for the next generation. A minimum of 12 cages per line were used for each generation, each female contributing equally to each cage (50 nymphs per cage). Two control lines were established using the same protocol as above except that females to be used as parents were selected at random. Due to an error the total fecundities of the two control lines were not measured in the second generation.

The low eggs laid selection experiment followed the above protocol except that the two replicates (selection line and control line) were initiated 3 weeks apart. In this case each control line can be matched with a particular selection line. Because counting eggs alone is much less labour intensive than measuring total fecundity it was possible to count the eggs laid by all adult females. We still selected the lowest 25 females which meant that in some generations the selection intensity was modestly higher than that used in the high total fecundity selection experiment.

Statistical analyses

There are three primary questions to be addressed: (1) Is there a direct response to selection? (2) What is the realized heritability of fecundity? (3) Is there a significant correlated response of wing morph frequency for both the high fecundity and low eggs laid selection experiments?

To address the first question we used two approaches. First, we used ANCOVA to analyse the change in fecundity over the generations of selection. To remove changes not directly associated with selection we subtracted the control means from the selected lines. In the case of the high total fecundity lines, because there is not a one to one match between control and selected lines, we subtracted the average of the two control lines

$$F^{*}_{i,j} = F_{i,j} - \left(\frac{\bar{x}_{1,j} + \bar{x}_{2,j}}{2}\right) \qquad (1)$$

where $F^{*}_{i,j}$ is the adjusted mean total fecundity of the ith selected line ($i = 1,2$) in the jth generation, $F_{i,j}$ is the mean total fecundity of the ith selected line in the jth generation, and $x_{k,j}$ is the mean fecundity of the kth control line ($k = 1, 2$) in generation j. Because of a lack of the correction for the control line, generation 2 was excluded from the analysis of the high total fecundity lines. For the low eggs laid line the adjusted fecundity was calculated by subtracting the mean value of the appropriate control line.

The second method of testing for a direct response to selection consisted of comparing the fecundity of control and selected-line females in the final generation. For the high total fecundity experiment we gave each line a unique designator and then tested for significant variation among the means with a one-way ANOVA, following this with a Tukey HSD multiple comparison test to locate the sources of variation. For the low eggs laid selection experiment we used a two-way ANOVA because control and selected lines could be paired.

We estimated the realized heritability as twice the slope of the regression forced through zero of the adjusted response on the adjusted cumulative selection differential (the slope is multiplied by two because selection is applied only to females). In the high total fecundity line we included the second generation by making the conservative assumption that there was no change in total fecundity in the selected lines. The standard error of the heritability was estimated using the formula given by Hill (1972) which takes into account drift,

$$Var(h^2) = \frac{6}{t(t+1)(2t+1)}\left(\frac{S_{\text{cum}}}{t}\right)^{-2} \left(\frac{2t^2+2t+1}{5}Var(drift) + Var(error)\right)$$

where (2)

$$Var(drift) = h^2 V_{\text{P}}\left(\frac{1-h^2}{N} + \frac{1}{L}\right)$$

$$Var(error) = V_{\text{P}}\left(\frac{1-h^2}{M} + \frac{h^2}{K}\right)$$

where L and N are the effective number of parents used per generation in the control and selected lines (= twice the number of actual individuals, because each female contributes the same number of offspring), respectively; K and M are the effective number of individuals measured per generation in the control and selected lines, respectively; t is the number of generations of selection; V_{P} is the phenotypic variance; S_{cum} is the adjusted cumulative selection differential.

As a second method of estimating the standard error we computed the standard error using the estimates of realized heritability from the two selection lines (Falconer, 1989, p. 211):

$$Var(h^2) = \frac{1}{2}\sum_{i=1}^{i=2}\left(h_i^2 - \frac{h_1^2 + h_2^2}{2}\right)^2. \qquad (3)$$

To analyse the correlated response of wing morph frequency we used the proportion of macropterous females per cage as individual data points. The correlated response should be measured as the change in the liability, which can be estimated as the abscissa of the standard normal curve corresponding to the observed proportion macroptery (Roff, 1994c). However, in some cases the proportion macroptery was zero, precluding the use of this transformation. For the purposes of testing for

a deviation between control and expected lines we therefore used the arcsine square-root transformation of the individual proportions. This procedure is valid for testing the deviation but gives only a crude measure of the actual shift in the liability.

We tested for a change in proportion macroptery as a function of treatment (selected or control) and generation. Because there is a nonlinear relationship between the value of the liability and the observed proportion we could not adjust the values in the selected lines by subtracting the control line values. Instead, after finding no significant variation between replicate lines within each experiment, we used the mean transformed values per generation, P_{trans}, in the covariance model,

$$P_{trans} = c_0 + c_1 t + c_2 TREAT + c_3(t)(TREAT) + error \quad (4)$$

where t is generation, *TREAT* is a categorical variable designating treatment (selected or control), and $c_0, \ldots, c_3$ are fitted constants.

Results

Direct response to selection

In both the high total fecundity and low eggs laid lines there was a clear deviation from the control lines in the direction expected as a result of selection (Fig. 1). The ANCOVA analysis indicated that there was no significant interaction ($F_{1,6} = 1.71$, $P = 0.24$, and $F_{1,4} = 0.18$, $P = 0.70$, for the high total fecundity and low eggs laid lines, respectively), no significant effect attributable to replicate ($F_{1,7} = 0.83$, $P = 0.39$, and $F_{1,5} = 3.37$, $P = 0.13$, respectively), but a highly significant change in fecundity with generation ($F_{1,7} = 15.3$, $P = 0.0058$ and $F_{1,5} = 16.4$, $P = 0.0098$, respectively: note that the analysis is sequential, the additive terms being tested after deletion of the nonsignificant interaction terms).

One-way analysis of variance of total fecundity in the last generation of the high total fecundity selection experiment indicated highly significant variation among lines ($F_{3,386} = 46.47$, $P < 0.00005$). The Tukey test showed no significant difference between the two control lines ($P = 0.99$), but all other comparisons were highly significant ($P < 0.001$). Two-way analysis of variance of fecundity in the last generation of the low eggs laid selection experiment gave a nonsignificant interaction ($P = 0.93$) between replicate and treatment (control vs. selected), but significant effects due to both treatment ($F_{1,394} = 20.44$, $P < 0.00005$) and replicate ($F_{1,394} = 6.25$, $P = 0.0128$). The two foregoing analyses indicate that selection was successful in changing fecundity in the direction intended.

Estimation of the realized heritability

The realized heritabilities of total fecundity in the high total fecundity selection experiment and eggs laid in the low eggs laid experiment were similar, ranging from 0.14 to 0.34, and were all significant (Table 1). Statistical difference between the estimates from the two replicate lines was tested using the model

$$R = c_0 S_{cum} + c_1 REP^* S_{cum} + error \quad (5)$$

where R is the response, S_{cum} is the cumulative selection differential, *REP* is a variable designating the replicate line (coded as 0,1), and c_0, c_1 are fitted constants. For the reason given earlier (see Materials and methods) the equation is forced through the origin and hence there is no constant or additive term for *REP* in the above equation. A significant interaction would indicate that the heritability estimates ($= 2 \times$ Slope) are significantly different. In neither experiment was the interaction term significant (for the high total fecundity experiment, $F_{1,8} = 1.86$, $P = 0.21$; for the low eggs laid experiment, $F_{1,4} = 5.66$, $P = 0.08$). The heritabilities from the combined analysis are very similar to the averaged values and both regressions are highly significant (Table 1).

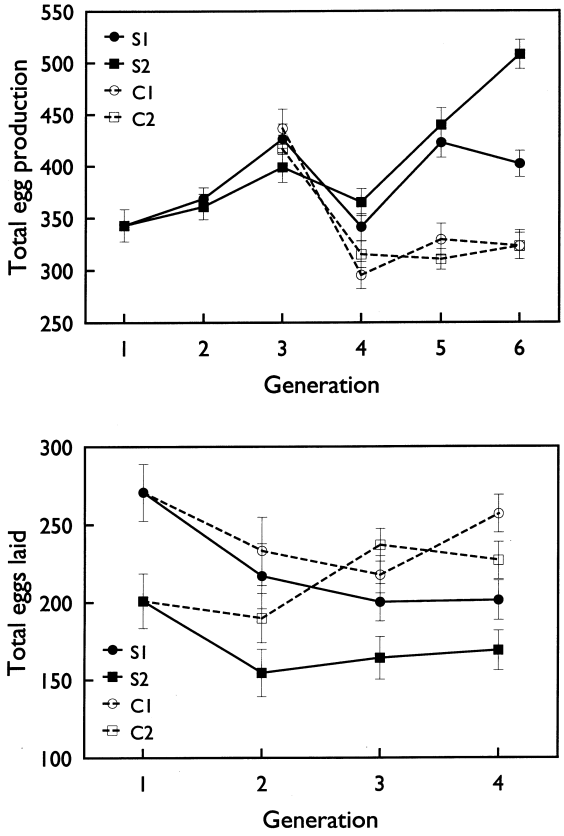

Fig. 1 Direct response to selection for increased total fecundity (top panel) and decreased number of eggs laid (bottom panel) in *G. firmus*. Solid line: selected lines; dashed lines: control lines.

Correlated response of wing morph frequency

The proportion of macropterous females decreased in lines selected for high total fecundity and increased in the

Table 1 Realized heritability estimates of fecundity in *Gryllus firmus.*

Type of selection	Replicate	Heritability	Standard error*	†*P*
High total fecundity	1	0.2	0.05 (0.04)	0.006
	2	0.34	0.06 (0.09)	0.0097
	average‡	0.27	0.07	–
	combined§	0.27	0.06 (0.05)	0.0003
Low eggs laid	1	0.14	0.04 (0.03)	0.0233
	2	0.30	0.06 (0.06)	0.0206
	average‡	0.22	0.08	–
	combined§	0.20	0.05 (0.05)	0.0035

*The standard error outside the parentheses was calculated using the formula of Hill (1972), that inside the parentheses is that obtained directly from the regression. †Probabilities are one-tailed. ‡The average heritability is the mean of the two realized heritability estimates with the standard error estimate estimated using eqn 3. §Estimate made by using both replicates in the same regression. The standard error estimated using the Hill (1972) formula was based on the mean values from the two replicate lines.

lines selected for low eggs laid (Fig. 2). In the high total fecundity experiment there was a significant decline in proportion macroptery over the course of selection and a significant difference between control and selected line but there was no significant interaction (Table 2). Thus the control line differed significantly from the selected line but this difference did not increase over generations, as expected. The lack of a significant interaction in the high total fecundity experiment is a consequence of the decline in the control line (Fig. 2). This generational change in the control line makes interpretation of the results ambiguous, although the significant treatment effect does suggest that there was at least a small correlated response

There was a significant interaction between treatment and generation in the low eggs laid selection experiment (Table 2) indicating, as would be expected, that the control and selected lines diverged in the proportion macropterous.

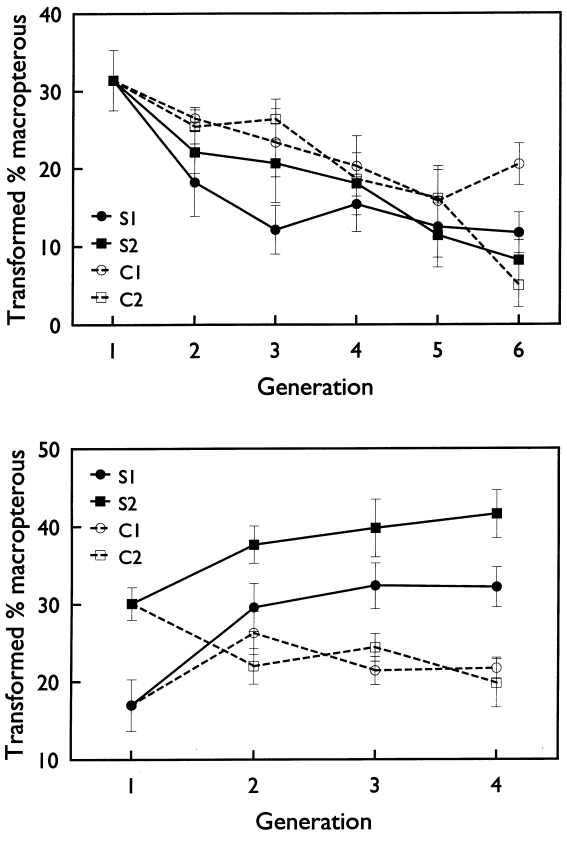

Fig. 2 Correlated response of proportion macroptery to direct response to selection for increased total fecundity (top panel) and decreased number of eggs laid (bottom panel) in *G. firmus.* Solid line: selected lines; dashed lines: control lines.

Is the change in fecundity a consequence of a change in proportion macroptery?

A change in the mean fecundity in the selected lines could be the result of two processes: response in fecundity within wing morphs, and/or a change in the proportion of macropterous females. To assess the relative importance of these two processes we examined fecundity in the final generation and changes in fecundity within morphs over the generations. Both analyses gave the same qualitative answer and we present only the analyses based on the final generation.

We tested for variation among micropterous females in the high total fecundity selection experiment in the same manner as for total fecundity (one-way ANOVA, followed by a Tukey test). In the two control lines micropterous females produced an average of 323 and 329 eggs, whereas the micropterous females from the selected lines produced 413 and 511 eggs. As with total fecundity, there is highly significant variation among groups ($F_{3,363} = 48.04$, $P < 0.00005$), no difference between the control lines ($P = 0.94$), and highly significant differences between all other pair-wise comparisons ($P < 0.0004$).

Table 2 Analysis of correlated changes in wing morph frequency in *Gryllus firmus*.

Source	d.f.	Mean square	*F*	*P*†
High total fecundity selection‡				
Generation	1	360.27	29.51	<0.00002
Treatment	1	112.97	9.25	0.0037
Error	17	12.21		
Low eggs laid selection				
Generation	1	0.34	0.01	0.4625
Treatment	1	30.26	0.84	0.1907
Generation * Treatment	1	150.4	4.16	0.0343
Error	10	36.11		

†Because there are *a priori* predictions, tests are one-tailed. †The interaction term was nonsignificant ($P = 0.36$) and was dropped from the model.

There were too few macropterous females to use the same method of analysis as for micropterous females: in this case we combined the replicate lines and tested for a difference with a *t*-test. Although macropterous females from the selected lines produced more eggs than macropterous females from the control lines (for the selected lines, $\bar{x} = 303$, $n = 12$, and for the control lines $\bar{x} = 235$, $n = 8$), the difference was not significant ($t = 0.84$, d.f. $= 18$, $P = 0.20$, one-tailed test).

For the low eggs laid selection experiment we analysed the data for each morph separately using a two-way ANOVA, as previously done for the population means. There were no significant effects due to replicate or the interaction between treatment and replicate ($P > 0.1$ in all cases) and so differences between treatments were analysed using a two-way ANOVA with treatment and morph as factors. The interaction was not significant and was therefore dropped from the model ($P = 0.17$). Both remaining terms were highly significant (for treatment, $F_{1,394} = 9.6$, $P = 0.0020$; for wing morph, $F_{1,394} = 33.10$, $P < 0.0005$). Macropterous females from the control lines laid an average of 199 eggs whereas macropterous females from the selected lines laid only 135 eggs. A similar difference was observed in the micropterous females, control line females laying 253 eggs and females from the selected lines laying 226 eggs.

The above analyses demonstrate that selection altered the population mean fecundity by changing the fecundity of the wing morphs, not by simply changing their relative frequency in the population. To assess the relative contribution of these two factors to the change in mean fecundity we proceeded as follows; first we computed the 'initial' mean fecundity, F_0, as

$$F_0 = p_0 F_{C,L} + (1 - p_0) F_{C,S} \quad (6)$$

where p_0 is the proportion of macropterous females in the first generation, $F_{C,L}$, $F_{C,S}$ are, respectively, the observed fecundity of macropterous and micropterous females from the final generation of the control lines (we used the final generation fecundity of the control line because we are comparing the fecundity of the selected and control lines at this generation: use of the initial fecundities did not make any significant change to the analysis). The fecundity predicted, F_P, assuming only a change in proportion macroptery from p_0 to p, and not within morphs is thus

$$F_P = pF_{C,L} + (1 - p)F_{C,S}. \quad (7)$$

The difference between F_P and F_0 is the component due to a change in proportion macroptery, and the difference between F_P and the observed fecundity in the selected line (F_{Obs}) is the component due to a change within morphs. The percentage change due to a change in proportion macroptery is thus given by $\frac{|F_P - F_0| \times 100}{F_0}$, and the percentage change due to a change within morphs is given by $\frac{|F_P - F_{Obs}| \times 100}{F_0}$. For the high total fecundity line the changes due to a change in proportion macroptery are 9% and 10%, while the changes due to within-morph increases in fecundity are 26% and 61%. For the low eggs laid lines the respective changes are 4%, 3% versus 17% and 20%. Thus the changes in mean fecundity were primarily the result of changes in fecundity within morphs.

Discussion

Selection on fecundity in either direction was successful in producing a significant direct response: in the high total fecundity lines total egg production increased by over 23%, and in the low eggs laid lines it decreased by over 22%. In the high total fecundity selection lines micropterous females showed an average 40% increase in fecundity ([Selected line mean fecundity – Control line mean fecundity]*100/Control line mean fecundity), whereas the macropterous females increased their fecundity by 27%. In the low eggs laid selection experiment micropterous females decreased their egg production by 11%, whereas the macropterous females showed a 31% decrease in eggs laid.

The realized heritability of total egg production in the first week of adult life (0.27) is similar to that estimated from half-sib analysis (0.20, sire estimate; 0.35, dam estimate; 0.27, combined; Roff *et al.*, 1997b). The higher dam estimate suggests the presence of dominance

variance, which has been confirmed by the observation of considerable inbreeding depression in fecundity (Roff, 1998). An earlier full-sib estimate of total egg production gave a higher value (0.65, SE = 0.16; Roff, 1994c). Full-sib estimates of heritability contain in their numerator one half of the dominance variance; after correcting for this using the half-sib analysis, the narrow sense heritability of fecundity is 0.47. This is larger than the present estimate, but given the confidence limits (0.15–0.39 for the realized heritability and 0.15–0.79 for the corrected full-sib estimate) the difference is not statistically significant.

Analysis of half-sib data for eggs laid in the first week gave a low sire estimate (0.07, SE = 0.08) and a low dam estimate (0.18, SE = 0.10; unpublished data). The large standard errors do not permit an assessment of nonadditive effects, but the similarity between the realized heritability (0.20) and dam estimate (0.18) argues for primarily additive genetic variance.

In both selection experiments there was a correlated response in the proportion of macropterous females. The extent of the correlated response can be estimated using the standard formulae (Falconer, 1989)

$$\begin{aligned} R_{\mathrm{F}} &= i h_{\mathrm{F}}^2 \sqrt{V_{\mathrm{PF}}} \\ CR_{\mathrm{M}} &= i r_{\mathrm{G}} h_{\mathrm{M}} h_{\mathrm{F}} \sqrt{V_{\mathrm{PM}}/V_{\mathrm{PF}}} \end{aligned} \tag{8}$$

where R_{F} is the direct response of selection on fecundity, i is the intensity of selection, h_{F}^2 is the heritability of fecundity, h_{M}^2 is the heritability of wing morphology (= heritability of the liability), V_{PF} is the phenotypic variance of fecundity, V_{PM} is the phenotypic variance of wing morph, which by definition equals 1 (Roff, 1994c), CR_{M} is the correlated response of wing morph (on the underlying scale), and r_{G} is the additive genetic correlation between fecundity and wing morph. Rearranging the above to eliminate the intensity of selection gives

$$CR_{\mathrm{M}} = r_{\mathrm{G}} R_{\mathrm{F}} \frac{h_{\mathrm{M}}}{h_{\mathrm{F}}} \sqrt{\frac{V_{\mathrm{PM}}}{V_{\mathrm{PF}}}}. \tag{9}$$

The above equation gives the change in the liability from which the change in the proportion macroptery is readily calculated using the standard normal distribution.

To calculate the predicted correlated change in proportion macroptery using the above equation we used the realized heritability estimates of fecundity and the phenotypic variances in fecundity from the control line females. We obtained all other estimates from a half-sib analysis (Roff *et al.*, 1997; unpublished data). For the high total fecundity experiment the proportion of macropterous females is predicted to change from its initial value of 0.30 to 0.08. The observed values (0.04 and 0.02) are similar to that predicted but the decrease in the control lines confounds the comparison. For the low eggs laid lines the predicted changes are (initial → final, observed): line 1, 0.19 → 0.32, 0.36; line 2, 0.37 → 0.49, 0.56. The predicted changes slightly underestimate the observed.

To analyse changes over a larger range in fecundity changes we calculated the predicted change in proportion macropterous for a change in total fecundity from 50 to 600 eggs and for eggs laid from 50 to 400 eggs (Fig. 3). Selection for a change in fecundity can dramatically alter the proportion of macropterous females in the population: for example, a change in total fecundity from the observed value of 343 eggs to 50 eggs is predicted to increase the proportion of macropterous females from 30% to 96%, while a decline to 50 eggs laid shifts the proportion macropterous to ≈70% macroptery (Fig. 3). The observed changes in proportion macroptery tend to be somewhat greater than predicted but show the same general trend (Fig. 3).

Because of the genetic and phenotypic correlation between trait values, the mean values of fecundity within morphs cannot be computed directly from the above equations. To obtain these values we modified the model given in Roff & Preziosi (1994), correcting the output to the appropriate scale. For each predicted fecundity–morph combination we generated 5000 individuals using the algorithm,

$$\begin{aligned} X_i &= \mu_x + a_{x,i}\sqrt{\frac{1}{2}h_{\mathrm{M}}^2} + b_{x,i}\sqrt{1-\frac{1}{2}h_{\mathrm{M}}^2} \\ Y_i^* &= r_{\mathrm{G}} a_{x,i}\sqrt{\frac{1}{2}h_{\mathrm{F}}^2} + a_{y,i}\sqrt{\frac{1}{2}(1-r_A^2)h_{\mathrm{F}}^2} \\ &\quad + r_{\mathrm{E}} b_{x,i}\sqrt{1-\frac{1}{2}h_{\mathrm{F}}^2} + b_{y,i}\sqrt{\left(1-\tfrac{1}{2}h_F^2\right)\left(1-r_E^2\right)} \\ Y_i &= Y_i^*\sqrt{V_{PF}} + \mu_y \end{aligned} \tag{10}$$

where:

X_i is the liability for the ith individual. The threshold value is set at 1 and the mean value in the initial population set such that the proportion of macropterous females, p, is the same as that observed. The threshold value remains constant but the mean liability, μ_x, changes as a result of selection. If individual i exceeds the threshold then it is designated a macropterous morph, otherwise it is the micropterous morph.

Y_i is the value of the fecundity for the ith individual.

$a_{x,i}$, $a_{y,i}$, $b_{x,i}$ and $b_{y,i}$ are random standard normal values, $N(0,1)$,

μ_y is the mean value of fecundity in the population after selection,

r_{E} is the environmental correlation, given by

$$r_{\mathrm{E}} = \frac{r_{\mathrm{P}} - \frac{1}{2} r_{\mathrm{G}} \sqrt{h_{\mathrm{M}}^2 h_{\mathrm{F}}^2}}{\sqrt{\left(1-\frac{1}{2}h_{\mathrm{M}}^2\right)\left(1-\frac{1}{2}h_{\mathrm{F}}^2\right)}} \tag{11}$$

where r_{P} is the phenotypic correlation.

As found from the statistical comparisons of fecundity within morphs, as the mean population fecundity (total or eggs laid) changes then so also does fecundity within

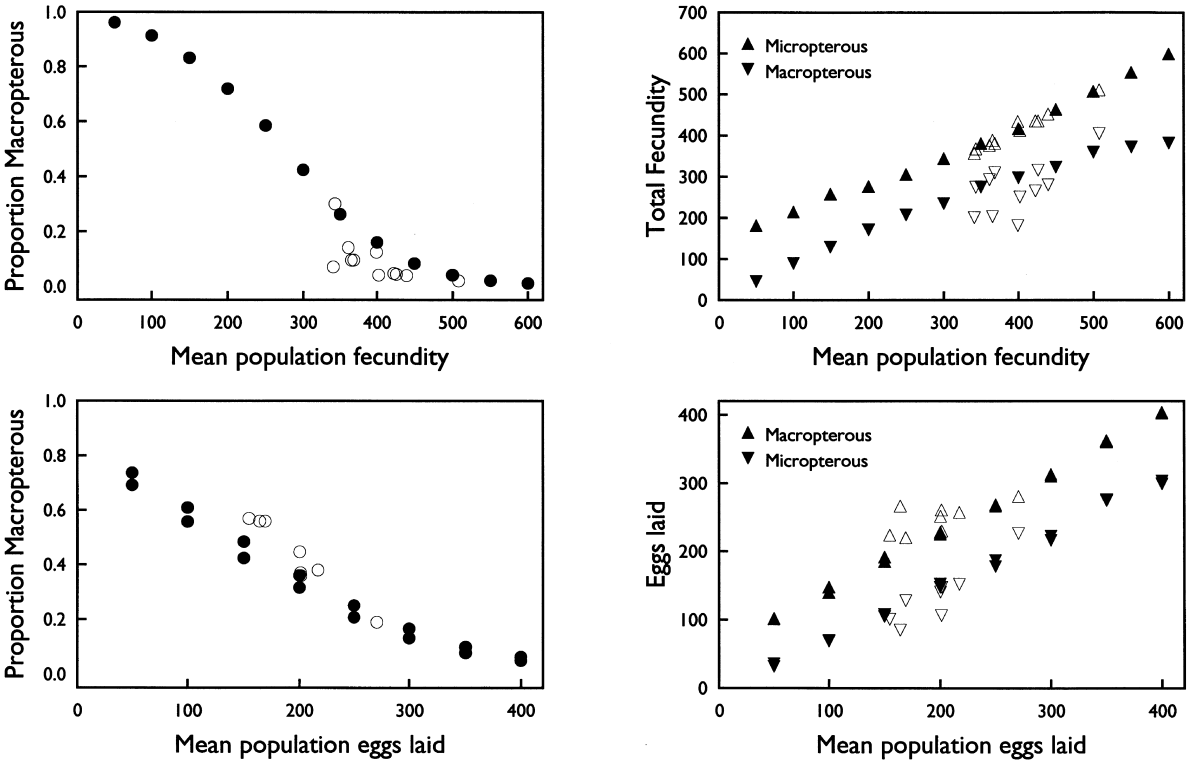

Fig. 3 Predicted (solid symbols) and observed (open symbols) direct and correlated responses to selection on fecundity. The *x*-axis shows the fecundity after selection, the initial fecundity being set at that observed in the selection experiments.

each morph, the difference in fecundity remaining approximately constant (Fig. 3; in the case of total fecundity the lines tend to diverge at extreme fecundities). Because the relative fitness of each morph is equal to the ratio of their fecundities (other life history components being kept constant), a constant difference implies a changing relative fitness (Fig. 4). For a fecundity greater than 250 eggs the relative SW fitness (micropterous fecundity/macropterous fecundity) is ≈1.4, but increases markedly below 250 eggs (Fig. 4). Thus selection on fecundity will change proportion macroptery by two processes: firstly, there will be a correlated response and secondly, the relative fitness of the two morphs itself changes with the mean fecundity. The latter process will be particularly strong when fecundity is less than 250 eggs.

There are important differences between the correlated responses observed for selection acting directly on proportion macroptery versus selection acting directly on fecundity. In the first case direct selection produced a direct response in the proportion macroptery but only a statistically significant correlated response in the fecundity of one morph (macropterous females: Roff, 1994c). Selection on fecundity, however, produced direct responses in fecundity within both wing morphs and a correlated change in the proportion macroptery (present paper). This difference in correlated responses can be accounted for by a model that incorporates a nonlinear pattern of resource allocation between the two wing morphs (see Appendix). Specifically it is postulated that

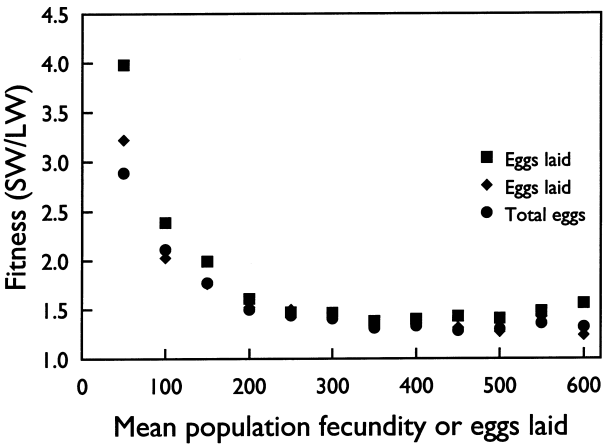

Fig. 4 Relative fitness of micropterous females as a function of the fecundity (total eggs or eggs laid: the two different symbols for the latter refer to the two replicates from the selection experiment) after selection, the initial fecundity being set at that observed in the selection experiments. Fitness is calculated as SW/LW (= micropterous fecundity/macropterous fecundity), where the fecundities are as shown in Fig. 3.

there is a trade-off between the maintenance of flight muscles and egg production: because micropterous females almost always have only very small flight muscles their fecundity cannot be radically changed by changing the age schedule of flight muscle histolysis as can and does occur in macropterous females (Fairbairn & Roff, 1990; Roff, 1994c). However, a change in the fecundity of the macropterous morph as a result of selection on macroptery will produce a change in the fecundity of the micropterous morph because it will change the relative fitnesses of the two morphs (Roff, 1994c). To understand the evolutionary dynamics of fecundity and wing morph requires a model that directly incorporates the functional architecture and the changing trade-off relationship. These results reinforce the conclusion reached by Riska (1986, 1989) and Houle (1991) that the analysis of functional architecture is important for an understanding of quantitative genetic variation and its relation to evolutionary change.

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Appendix

The following model demonstrates how differences in correlated responses can arise when one of the component traits is a threshold trait. We shall use wing dimorphism and fecundity as example traits.

Fecundity is assumed to be influenced in two ways by pleiotropic effects of the liability genes: one effect is directly due to the liability genes, and corresponds to the usual genetic correlation between traits. A second effect is due to the 'competition' for resources between the flight muscles and the gonads. In the latter case the pleiotropic effects of the liability genes are produced via the threshold response, because the effect will be felt almost solely in the macropterous females, micropterous females having no or very small muscles (Roff, 1989; Fairbairn & Roff, 1990; Zera *et al.*, 1997). For simplicity of discussion we shall assume that the two pleiotropic effects are uncorrelated. Fecundity can now be decomposed into two components

$$F = X - A \tag{A1}$$

where X is the fecundity component that is correlated with liability alone and A is the fecundity lost from allocation to flight muscle maintenance. Assuming, without loss of generality, that macropterous females are those that lie above the threshold (large liabilities), the genetic correlation between the liability and X is negative, whereas the genetic correlation between the liability and A is positive (i.e. large liabilities lead to increased commitment to maintenance of flight muscles and hence reduced fecundity).

First consider selection on proportion macroptery: the correlated response of fecundity is given by

$$CR_F = i_M\sqrt{V_{PF}}h_M(h_X r_{MX} + h_A r_{MA}) \tag{A2}$$

where V_{PF} is the phenotypic variance in fecundity, r_{MX} is the genetic correlation between X and the liability (macroptery), and r_{MA} is the genetic correlation between A and the liability. Now if the product $h_X r_{MX}$ is small, the correlated response in fecundity will come about primarily by a change in allocation: consequently the correlated change in fecundity will occur primarily in macropterous females, as has been observed (see Introduction).

To predict the correlated responses to selection on fecundity we must consider the correlations between the components of fecundity and fecundity itself. The problem is one of part-whole correlation (Sokal & Rohlf, 1995). Under the assumption of no correlation between X and A we have

$$r_{FX} = \sqrt{\frac{V_X}{V_X + V_A}}, \qquad r_{FA} = -\sqrt{\frac{V_A}{V_X + V_A}} \tag{A3}$$

and the genetic correlation between the liability and fecundity is

$$r_{MF} = \frac{Cov_{MX} + Cov_{MA}}{\sqrt{(V_{AX} + V_{AA})V_{AM}}} \tag{A4}$$

where *Cov* designates the covariance between the liability (M) and the two fecundity components (X, A). The direct response to selection on fecundity is given by

$$R_F = i_F \frac{V_{AX} + V_{AA}}{V_{PX} + V_{PA}}\sqrt{V_{PX} + V_{PA}}. \tag{A5}$$

And the correlated responses are

$$\begin{aligned} CR_X &= i_F\sqrt{\frac{V_{AX}}{V_{AX} + V_{AA}}}h_X h_F\sqrt{V_{PX}} \\ CR_A &= i_F\sqrt{\frac{V_{AA}}{V_{AX} + V_{AA}}}h_A h_F\sqrt{V_{PA}} \\ CR_M &= i_F\frac{Cov_{MX} + Cov_{MA}}{\sqrt{(V_{AX} + V_{AA})V_{AM}}}h_M h_F\sqrt{V_{PM}} \end{aligned} \tag{A6}$$

For direct selection on fecundity to alter fecundity within morphs we require a large correlated response in X. Recall that the product $h_X r_{MX}$ is small: for the correlated response in X, CR_X, to be large we require h_X to be relatively large. Therefore, r_{MX} must be small, which requires Cov_{MX} to be small and/or V_{AX} to be large. The magnitude of Cov_{MX} does not affect CR_X while the

effect of an increase in V_{AX} on CR_X depends on the value of V_{AX} relative to V_{AA}. The correlated response in allocation also does not depend on Cov_{MX} and could be large or small independently of the correlated response in X.

Finally, we must consider the correlated response of macroptery to selection on fecundity. Even if, as suggested above, Cov_{MX} is small the correlated response in macroptery can be large if Cov_{MA} is large.

Thus if the genetic covariance between the liability and the fecundity component correlated directly with the liability (Cov_{MX}) is small then (i) direct selection on proportion macroptery will produce a change in macroptery and a correlated response in fecundity primarily in the macropterous morph, and (ii) direct selection on fecundity will produce a direct response in fecundity in both morphs and a correlated response in proportion macroptery.