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Influence of genetic architecture on contemporary local evolution in the soapberry bug, *Jadera haematoloma:* artificial selection on beak length

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Abstract

Little is known about the influence of genetic architecture on local adaptation. We investigated the genetic architecture of the rapid contemporary evolution of mouthparts, the flight polymorphism and life history traits in the soapberry bug *Jadera haematoloma* (Hemiptera) using laboratory selection. The mouthparts of these seed-feeding bugs have adapted in 40–50 years by decreasing in length following novel natural selection induced by a host switch to the seeds of an introduced tree with smaller fruits than those of the native host vine. Laboratory selection on beak length in both an ancestral population feeding on the native host and a derived population feeding on the introduced host reveals genetic variance allowing a rapid response (heritabilities of 0.51–0.87) to selection for either longer or shorter beaks. This selection resulted in reverse evolution by restoring long beaks in the derived population and forward evolution by re-creating short beaks in the ancestral bugs. There were strong genetic correlations (0.68–0.84) in both populations between beak lengths and the frequency of flight morphs, with short beaks associated with short wings. The results reveal a genetically interrelated set of adaptive multivariate traits including both beak length and flight morph. This suite of traits reflects host plant patchiness and seeding phenology. Weaker evidence suggests that egg mass and early egg production may be elements of the same suite. Reversible or forward evolution thus may occur in a broad set of genetically correlated multivariate traits undergoing rapid contemporary adaptation to altered local environments.

Introduction

The phenomenon of local adaptation reveals the power of natural selection and other processes that lead to the evolution and divergence of populations connected by gene flow (Kawecki & Ebert, 2004). In many cases such evolution can take place in a few decades of 'ecological time' (Hairston *et al.*, 2005). Such rapid contemporary evolution of local populations is particularly apparent in organisms of short generation time like insects (e.g. Carroll & Boyd, 1992; Singer *et al.*, 1993; Majerus, 1998), but there are many instances where it occurs in fewer than tens of generations in long lived organisms from fish to trees (e.g. Hendry & Kinnison, 1999; Strauss *et al.*, 2006; Herrel *et al.*, 2008). Local adaptation offers excellent opportunities to study links between traits under selection and the agents of selection and their strength (Kawecki & Ebert, 2004). This places a premium on the ability to identify selective agents. An emerging insight is that profound adaptive local genetic change can take place rapidly in response brought about by species invasions, which offer the advantage of selective agents often readily identifiable (O'Dowd *et al.*, 2003; Lambrinos, 2004; Sax *et al.*, 2007; Carroll, 2008). We are beginning to understand the architecture underlying local adaptation resulting from both additive and non-additive genetic influences (Bradshaw & Holzapfel, 2001; Bradshaw *et al.*, 2005; Roff & Mousseau, 2005; Orr, 2005;

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Carroll, 2007; Carroll *et al.*, 2001). In particular natural selection acts on suites of traits likely to be genetically correlated, and selection can act on both the strength and direction of the genetic correlations as well as on individual traits (Bradshaw, 1986; Roff & Mousseau, 2005; Roff & Fairbairn, 2007).

We have been studying the local adaptation of the mouthparts (stylets or 'beaks') of the North American soapberry bug, *Jadera haematoloma*. The beaks of these true bugs evolve to match the size of the fruits they must penetrate to reach the seeds on which they feed (Carroll & Boyd, 1992). In several instances, the bugs have spread to introduced exotic hosts and have evolved beak lengths appropriate to the new host fruits in fewer than 100 generations or 40–50 years. In addition, populations on native and introduced hosts differ in a number of other metabolic, morphologic and life history traits (Dingle, 2001 and below). These bugs are thus good candidates for investigating the genetic changes occurring during rapid local adaptation including the possibility of genetic correlations among traits. In this article we investigate this evolution using laboratory selection on beak length in both ancestral and derived populations of soapberry bugs to explore direct and correlated responses to selection on beak length, the latter in traits that are known to differ between the ancestral and derived bugs.

We selected for both shorter and longer beaks in ancestral and derived populations to reveal the potential for reverse and continued forward beak length evolution after strong selection has produced marked local adaptation. Interest in the reversibility of evolution for particular phenotypes (i.e. the reacquisition of ancestral states) has a long history in evolutionary biology, but the extent to which it can occur over short time spans is still largely an open question (Teotónio & Rose, 2001). There are several examples of reverse evolution in experimental laboratory populations and evidence of selection acting on standing genetic variation (Teotónio *et al.*, 2009), but only a few examples for natural populations (Teotónio & Rose, 2001). Probably the best known case in nature is that of industrial melanism in the peppered moth, *Biston betularia*, where the melanic form increases in frequency in both Europe and North America in industrial areas, but the pale or peppered form is restored to high frequency when industrial air pollution is reduced (Grant *et al.*, 1996; Majerus, 1998), although the selective agents are not fully understood. In soapberry bugs evolution of beak length occurs in both forward and reverse modes because both longer and shorter beaks are known to evolve from ancestral populations depending on the size of host fruits (Carroll & Boyd, 1992). These insects provide an opportunity to assess reverse plus continued evolution in a case of rapid local adaptation where the source of selection – the characteristics of different species of host fruits – have been relatively clearly identified. Thus, in addition to examining the genetic architecture of local host adaptation (Carroll *et al.*, 1997 *et seq.*; Dingle, 2001), we now use laboratory selection to examine the potential for continued evolution of adaptive beak length to either larger or smaller seeds of native and introduced (or invading) hosts.

Local adaptation in soapberry bugs

Soapberry bugs (Hemiptera: Rhopalidae: Serinethinae) are a subfamily of three widespread genera of predators on seeds of the Sapindaceae or soapberry family. Two native North American hosts are soapberry tree (*Sapindus saponaria*) and balloon vine (*Cardiospermum halicacabum*) fed on by *J. haematoloma,* the subject of this paper. In North America and Australia the exotic Asian goldenrain trees, *Koelreuteria elegans* and *Koelreuteria paniculata*, have been introduced into urban areas beginning in the 1940s. Populations of *J. haematoloma* in North America and the Old World soapberry bug *Leptocoris* in Australia have colonized and evolved on these trees and continue to use native hosts as well (Carroll & Boyd, 1992; Carroll *et al.*, 1997, 2005). Goldenrain tree introductions thus provide an opportunity to observe ecological and evolutionary processes of local adaptation in real time, as noted by Sax *et al.* (2007). The introduced hosts differ from natives in fruit size (Carroll & Boyd, 1992), amount and phenology of fruit production (Carroll *et al.*, 2003a), and seed nutritional quality and chemistry (Siegler & Kawahara, 1976; Carroll *et al.*, 1998). Host characteristics have contributed towards selection for local adaptations in the bugs. The most apparent and dramatic diversification has been in beak length, which has evolved in 40–50 years to better match the fruit size of exotic hosts in both the American and Australian soapberry bugs (Carroll & Boyd, 1992; Carroll *et al.*, 2005; Carroll, 2007).

Our most extensive studies have been on Florida, USA populations of *J. haematoloma,* and these are the populations on which we report here. 'Ancestral' Florida Keys bugs have long beaks to penetrate the large fruits of the native balloon vine. In northern and central Florida a 'derived' population of bugs has evolved on the introduced flat-podded *K. elegans* and possesses much shorter beaks appropriate to the compressed fruit of this tree. Changes have also occurred in clutch size, egg mass, development time, body size and shape, host preference, the relative frequency of morphs in a wing and flight muscle polymorphism (described below) (Dingle, 2001; Carroll *et al.*, 2003a), the response of this polymorphism to juvenile hormone and density (Dingle & Winchell, 1997), and levels of metabolic enzymes in flight muscle (Winchell *et al.* 2001). Within and between population crosses indicate that these differences have resulted from changes in genetic architecture, including significant epistasis (Dingle & Winchell, 1997; Carroll *et al.*, 2001, 2003b). Fundamental questions that we address are whether and to what extent these traits have evolved together or independently in adapting to local environments and whether evolution can be ongoing or reversed.

Materials and methods

Rearing

Bugs from the derived and ancestral populations were collected from two sites in Florida, based on the distribution of the host races. The ancestral race was collected from the native balloon vine on Key Largo (25.05°N Lat, 80.27°W Long) and Plantation Key (24.70°N, 80.33°W) in the Florida Keys. The derived population was collected from goldenrain trees (*K. elegans*) in suburban neighbourhoods in the town of Leesburg in north central Florida (28.19°N, 81.53°W). The geographical separation of sites (450 km) was such that gene flow between them would likely be absent or extremely rare given the flight capacities and behaviour of the bugs (Carroll & Boyd, 1992; Carroll *et al.*, 2003a,b).

About 200 freshly collected insects from each population were brought to the laboratory and mass reared through one generation in environmental chambers at 31 °C and LD 14 : 10 to approximate ambient conditions at both source areas. They were fed *ad libitum* on the seeds of the host plants from which they were collected and provided distilled water from cotton-stoppered vials. In these stock cages cardboard egg containers provided additional surface area, and a thin layer of plaster-of-Paris mixed with charcoal powder to absorb waste covered the bottom. Bugs readily laid eggs on this surface.

Mated pairs in the selected and control lines were reared in individual 90 mm Petri dishes with filter paper on the bottom and a water vial. Goldenrain tree seeds were supplied for food (either *K. elegans* or similarly suitable *Koelreuteria bipinnata*, Carroll, 2007) because we were exploring the 'evolutionary path' of evolving populations on these exotic hosts (see above). Females readily laid eggs on the bottoms of the dishes from whence they were collected by aspiration to minimize handling damage. Otherwise all rearing was performed in the same environment and ambient photoperiod and temperature were as above throughout the duration of the selection experiment. Approximately 30 eggs were collected from each pair, placed into a separate container, and reared through to adult eclosion, at which point selection was performed to establish the next generation (see below).

Selection

Two replicated lines selected for long beaks, two replicated for short beaks and two randomly mated control lines were established for each population (ancestral and derived). Lines were generated by first randomly choosing forty females and forty males from the mass cultures of each host race. These were then randomly paired and randomly assigned to two groups of 20 pairs each that served as the founders of the control (unselected) lines. In subsequent generations there was randomization of families within each control line. In each generation three offspring of each sex were randomly chosen from each parental pair and randomly mated with a member of the opposite sex from another family. One of these pairs was designated the primary, and the other two served as reserves in case a member of the primary pair did not produce a sufficient number of mature offspring. This process was repeated for each control replicate of each host race across all generations of selection. Similar procedures were followed for the replicate lines selected for upwards and downwards selection on beak length. In these cases, however, the three bugs of each sex with the longest or shortest beaks, depending on line, were chosen as parents for the next generation. In this 'within family' selection design each family is represented in the succeeding generation, rather than individuals being selected without regard to family. This design slows response to selection but has the advantage of reducing the loss of genetic variation that might be caused by drift or bottlenecking (Falconer & MacKay, 1996 and see Palmer & Dingle, 1986 and Dingle *et al.*, 1988 for use of within family selection with another seed-feeding bug). In this study we did find that response to selection was rapid and obvious. We measured beak lengths with hand held digital calipers with 0.01 mm measurement interval from the anterior tip of the tylus to the distal tip of the beak.

To assess genetic correlations with beak length, we recorded wing length (short vs. long – see below) throughout selection. In the final generation of selection we measured additional traits. In the latter case statistically significant differences between a trait measured in selected and control lines would indicate a correlated response to selection (and genes shared in common with beak length), although the exact value of the correlation cannot be determined. The traits measured include post-eclosion age at first egg production (interval between eclosion and first egg laid), egg mass, fecundity for the first 6 days of reproduction (number of eggs produced by a female in this interval), reproductive effort (mean egg mass times total eggs over the first 6 days), lifespan (days from eclosion to death), and behaviour (host seed preference). Clutches were weighed with a microbalance and the mass divided by egg number to obtain egg mass.

We assessed host preference in separate groups of naïve hatchlings from each family. The bugs usually feed in groups in nature, so host preference was tested in groups. Ten hatchlings from each family were placed in clear plastic boxes ($18 \times 12.5 \times 5$ cm high), and three seeds from each host were placed at opposite ends of the floor. For native balloon vine we substituted seeds of its close congener *C. halicacabum*, and for introduced goldenrain tree we used seeds of *K. bipinnata.* We recorded the number of individuals feeding on each host at three hourly intervals over a 9–12 h period each day until a maximum of 10 feeding episodes was obtained.

A complication throughout these analyses is that both populations of soapberry bugs exhibit a four morph flight polymorphism (Dingle & Winchell, 1997; Dingle, 2001; Carroll *et al.*, 2003a). The morphs are (1) a short-winged form with no flight muscles, and three long-winged forms, (2) one with no flight muscles, (3) one that histolyzes flight muscles several days after adult eclosion and upon feeding, and (4) one that retains flight muscles throughout life. Forms 1 & 2 are more frequent in natural populations from Leesburg, whereas forms 3 & 4 are more common in the Keys, Only the short-winged morph can be identified with certainty without dissection to determine presence or absence of flight muscle. Thus, in assessing correlations of beak length with flight morph, we could separate only long- from short-winged forms.

Statistics and estimation of genetic parameters

Basic statistical analyses were performed with the SAS System Mixed Procedure using the basic model population + line + replicate within line + pop*line + family. Modifications included wing morph in the model and wing morph and body size as covariates (see below for further comment on wing morph).

To evaluate selection response in beak length and flight morph, two potentially correlated traits, in individuals that are related through a common base population, separate analyses were conducted for the Keys and Leesburg populations, with the same statistical approach for each population. Beak length was evaluated as a continuous characteristic and wing morph as a binary trait. To estimate the genetic variances and covariances among these traits, we consider the binary trait of wing length to behave according to a threshold model of expression (Falconer & MacKay, 1996; Pulido, 2007; Roff & Fairbairn, 2007), an approach typical for the analysis of binary and ordered categorical traits. The model for the underlying and unobservable continuous variate is similar to any that can be used for continuous phenotypes. That model included terms for sex, selected line, replicate within selected line and the additive genetic contribution of each animal in the experiment. The additive genetic effect for each individual accounts for the covariance in phenotypes of relatives and is assumed to be multivariately normally distributed, with a covariance structure based upon the additive relationships among all animals, built from the known pedigree. Because the underlying scale is unobservable, the total variance is assumed to be the sum of the additive genetic and residual variances where the residual variance is set to 1.0 with no loss of generality (Sorensen *et al.*, 1995; Felsenstein, 2005).

Evaluating the trait of beak length is similar to that described for wing length; however, it is not necessary to presume an underlying continuous variate. Rather, we model beak length as a normally distributed phenotype that is a function of sex, selection line and replicate, along with a presumed additive genetic contribution to beak length that follows from the known pedigree of animals in the experiment.

Evaluating these two traits together permits estimation of the genetic and residual covariances across these two polygenic traits. To estimate the unknown fixed effects and unknown variances and covariances we used a mixed model Bayesian strategy. A more complete description of the statistical aspects of this analysis is available in Sorensen & Gianola (2002). Briefly, the assumed prior densities for the fixed effects (sex, selection line and replicate effects) are uniform. As for the random contributions to each trait, the additive genetic effects are assumed to be a multivariate-normal distribution with a zero mean and variance-covariance structure consisting of the numerator relationship matrix times the unknown additive genetic variances and covariances. Similarly the random residuals are assumed to be independent multivariate-normal distributions with zero means. The prior density for the unknown variances and covariances is the inverted Wishart distribution with a shape parameter of 5 (the minimum for a two-trait analysis being 4, Sorensen *et al.*, 1995). A value of 5, speaking relatively, would be considered small, reflecting weak prior knowledge of the actual value for the additive genetic parameters of these two traits. Preliminary analyses with the minimum value of 4 did not consistently converge to stable estimates of the genetic correlation; accordingly we used a value of 5 in subsequent analyses.

Estimation of the posterior distribution of the unknown parameters employs Gibbs sampling, a technique of numerical integration (Geman & Geman, 1984). A more complete description of the Gibbs sampling process and its theoretical justification is given by Van Tassel & Van Vleck (1996), as well as in the manual of the public domain software, MTGSAM, with which this analysis was performed.

In this study, the total length for the Gibbs sampling process was set to 300 000, with the first 50 000 samples discarded from any subsequent analysis, a set created from five independent chains of sampling. The post-Gibbs analysis was implemented with the packages boa (Smith, 2007) and coda (Plummer *et al.*, 2007), both part of the R-program (R Development Core Team 2005). Convergence of the Gibbs sampling process was evaluated visually by sample plots and by a diagnostic test contrasting sample means from the first 10% of the sample with the last 50% of the sample (Geweke, 1992). Autocorrelations were calculated within the complete Gibbs sample to arrive at a suggested thinning rate. Gibbs sample statistics were calculated with a thinning rate of 25 (chosen based on computation of a maximum autocorrelation at lag 25 of 0.02 for all parameters), creating a final Gibbs sample of 10 000

sample observations (i.e. [300 000–50 000]/25 = 10 000). Highest density regions (see Table 1) were computed as described (Hyndman, 1996) with public domain software hdrcde (Hyndman, 2008), a package within the R-program.

Results

Response to selection

A phenotypic response to direct selection on beak length was apparent in both the short-beaked Leesburg (derived) and the long-beaked Keys (ancestral-type) populations (Fig. 1). In Leesburg and the control and short selected Keys lines selection continues to the sixth generation. Delays in reproduction in the Keys lines selected for increased beak length resulted in only 4 and 5 generations in the two replicated long beak lines (Fig. 1c,d). These results indicate considerable additive genetic variance for beak length, confirming previous results (Carroll *et al.*, 2001), and that this genetic variation was not exhausted during the course of the selection applied here. Heritability estimates were 0.51 for the long-winged Keys lines and 0.60 for the brachypterous (short winged) Keys bugs (see below) and 0.68 and 0.87, respectively, for the Leesburg sample (Table 1). Previously we demonstrated using population crosses that there is also epistatic and dominance variance for beak length (Carroll *et al.*, 2001, 2003b).

Brachypters appeared with sufficient frequency during the course of selection to plot beak length generational means separately for this morph. Directional response to selection was apparent in these brachypters, but the responses from one generation to the next tended to be variable and not always in the expected direction. Sample sizes of brachypters were relatively small, leading one to suspect the possibility of genetic drift. Another factor, however, may be involved. Using a threshold model as described in the Methods, we estimated genetic correlations between beak length and wing morph (Table 1). For the Keys sample this was −0.68 and for Leesburg −0.84, both strongly negative. In other words, as beaks become longer the frequency of brachypters declines. The genetic architecture thus indicates that beak length and the frequency of wing morphs apparently share genes in common (Dingle, 2001), and selection on beak length will generate a response in the wing morph frequencies. It is possible that beak length selection also influences the frequencies of the morphs within the long-winged external morphology, i.e. the frequencies of morphs 2, 3 and 4 above, but this could not be determined here.

A further question concerned whether we could induce reverse evolution by selection on the derived short-beaked Leesburg bugs and thus a generation with mean beak length equivalent to that of the ancestral Keys population. Conversely could we duplicate forward evolution in short Leesburg beak lengths by selecting

Table 1 Parameter estimates calculated from results of selection for beak length.

	Mean	Median	SD	95% HDR Low	95% HDR High
Leesburg results					
Genetic Var LW	2268	2267	65.3	2140	2400
Genetic Var SW	7.04	6.71	1.66	4.24	10.85
Heritability LW	0.68	–	0.02	0.65	0.71
Heritability SW	0.87	–	0.02	0.83	0.92
Gen Correlation	−0.84	–	0.01	−0.86	−0.82
Phen Correlation	−0.72	–	0.01	−0.73	−0.70
Residual Corr	−0.36	–	0.04	−0.43	−0.28
Gen Cov LWSW	−105.0	−103.1	12.79	−133.8	−82.16
Residual Var LW	1066	1066	47.85	972.4	1160
Res Cov LWSW	−11.62	−11.63	1.38	−14.34	−8.90
Keys results					
Genetic Var LW	2603	2601	116.5	2379	2835
Genetic Var SW	1.51	1.49	0.21	1.11	1.49
Heritability LW	0.51	–	0.02	0.47	0.54
Heritability SW	0.60	–	0.03	0.53	0.67
Gen Correlation	−0.68	–	0.03	−0.72	−0.63
Phen Correlation	−0.68	–	0.01	−0.70	−0.66
Residual Corr	−0.70	–	0.02	−0.75	−0.65
Gen Cov LWSW	−42.26	−42.08	3.69	−49.69	−35.02
Residual Var LW	2527	2526	87.80	2357	2702
Res Cov LWSW	−35.04	−35.02	1.54	−38.18	−31.98

LW refers to data from the majority long-winged individuals and SW refers to separate estimates from short-winged individuals (see Methods). Correlations are between beak length and wing length. HDR values are the confidence limits around the means.

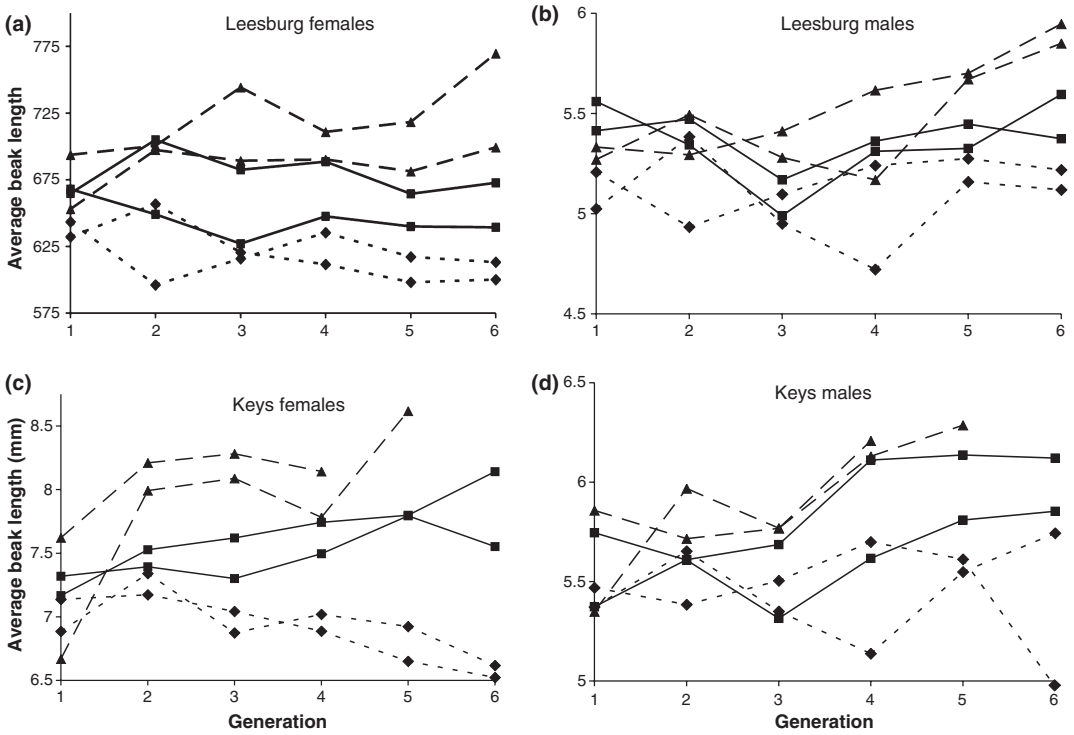

Fig. 1 Responses to within family bi-directional laboratory selection for beak length in derived Leesburg and ancestral Keys populations of soapberry bugs. Generational means for selection for longer beaks are joined graphically with dashed lines; control means are joined with solid lines, and short with dotted lines. Note that because of delays in reproduction, selection proceeded for only 4 or 5 generations in Keys long-beaked lines.

downward on the long-beaked Keys sample, the direction of beak length evolution in natural populations (Carroll & Boyd, 1992)? The affirmative answers are revealed in Table 2 which shows mean beak lengths for founder populations and for the final generation of all selected and control lines. Leesburg bugs selected for long beaks reached mean beak lengths of 7.35 and 5.90 mm for females and males respectively, compared with 7.35 and 5.70 mm in the founders of the Keys selected lines (i.e. Keys 'wild type'). Values for Keys control lines were 7.75 and 5.90 mm. Thus, short-beaked Leesburg lines reached long-beaked equivalency with ancestral Keys bugs after only five generations of within family laboratory selection.

Table 2 Mean (± SD) beak length (mm) comparisons of last generation selected and control lines of each population with mean beak lengths of all founders of these lines.

	Leesburg		Keys	
	Females	Males	Females	Males
Founders	6.60 ± 0.71	5.35 ± 0.39	7.35 ± 1.16	5.70 ± 0.88
Long	7.35 ± 0.76	5.90 ± 0.34	8.30 ± 0.64	6.20 ± 0.33
Short	6.10 ± 0.39	5.20 ± 0.31	6.60 ± 0.74	5.40 ± 0.54
Controls	6.85 ± 0.69	5.45 ± 0.44	7.75 ± 0.86	5.90 ± 0.39

Turning to the converse question, Keys lines selected for short beaks reached 6.60 and 5.40 mm for females and males compared with 6.60 and 5.35 mm and 6.85 and 5.45 mm for Leesburg founders and controls. The results of these selection experiments indicate that after 4–6 generations of laboratory selection respective beak length changes were comparable to those attained during some portion of a span of about 100 generations in the field (Carroll & Boyd, 1992) where long-beaked Keys ancestrals evolved to short-beaked Leesburg types, and further that the effect could be reversed in a similar number of generations of selection. Note that the size values in these laboratory populations, including those of beak length, averaged smaller than those in field populations (e.g. Carroll & Boyd, 1992). The point here is that, in the laboratory environment, 4–6 generations of reciprocal selection on long and short-beaked lines was sufficient to induce forward and reverse evolution and 'recreate' in each line the beak length value of the other.

Correlated responses

In addition to the genetic correlation between beak length and wing morph frequency, we found evidence of genetic correlations between beak length and two life history traits, egg mass and the first 6 days of egg

production. Results for egg mass are plotted in Fig. 2. Evidence of a genetic correlation is indicated by a difference between values for the control lines and those of selected lines. In the case of the Keys sample the difference between control and long lines was not statistically significant, but that between control and short was (t_{453} = 2.53, P = 0.012), suggesting a positive genetic correlation only when selection was applied for shorter beaks. A similar result was observed in the Leesburg population with no significant difference between control and long-beaked lines but strong evidence for a correlation in the short/control difference (t_{453} = 5.34, P < 0.0001).

There was possible evidence of a genetic correlation between beak length and first 6 day egg production in the Keys bugs but not in Leesburg (Fig. 3). In the Keys there was indication of a negative correlation between these traits, although only the difference between the control and long selected lines was significant (t_{442} = 4.18, P < 0.0001). In Leesburg there was a significant reduction in eggs produced in both long and short selected lines, suggesting causes other than genetic correlation were responsible for the differences. These remain unknown, as we noted no obvious differences among lines in the condition of the bugs or in mortality.

For the remaining traits examined no differences among selected and control lines were observed, and so there was no evidence supporting the presence of genetic correlations. The results for host preference in particular were nevertheless interesting because they reveal differences between the Leesburg and Keys populations in 'wild type' host preference (Fig. 4). The native host feeding Keys bugs, which are ancestral to central Florida populations like Leesburg (Carroll & Boyd, 1992) prefer the seeds of *Koelreuteria,* the introduced host, but by a smaller margin than the Leesburg bugs. The Leesburg bugs, which have been evolving on *Koelreuteria* hosts

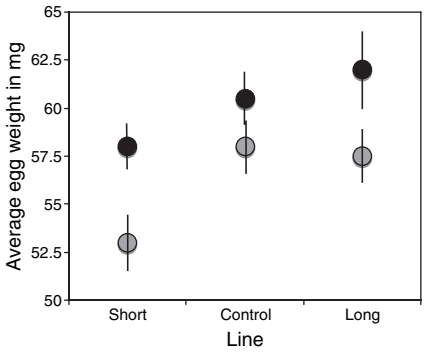

Fig. 2 Means (± SE) egg mass in the final generation of selection for both selected populations. Levels of significance (*t*-tests) as follows: Keys (black): control vs. long, n.s.; control vs. short, P = 0.012; Leesburg (grey): control vs. long, n.s.; control vs. short, P < 0.0001; Keys control vs. Leesburg control, P < 0.01.

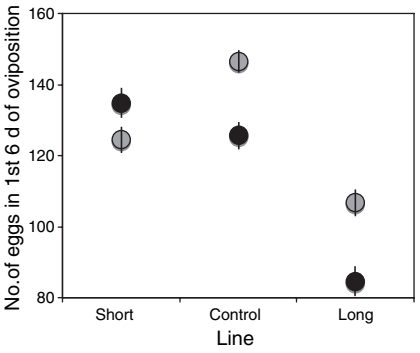

Fig. 3 Mean (± SE) number of eggs produced by individual females during the first 6 days of reproduction in the final generation in both selected populations. Levels of significance (*t*-tests) as follows: Keys (black): control vs. long, P < 0.0001; control vs. short, n.s.; Leesburg (grey): control vs. long, P < 0.0001; control vs. short, P < 0.004. Keys control vs. Leesburg control, P < 0.008.

greatly prefer these seeds on which they now exclusively feed and on which they have evolved their shorter beaks (Carroll & Boyd, 1992; Carroll, 2007). Because the introduced tree was an exploitable resource rather than a lethal evolutionary trap (see Carroll & Watters, 2008), the transfer to this host would have been straightforward. Once it occurred, selection by host differences in fruit size, fruiting phenology and seed nutritional quality engendered the evolution of a new set of adaptive trait values.

Discussion

The genetic variance for beak length present in both ancestral and derived populations of Florida soapberry bugs resulted in a rapid response in both forward and reverse directions to the within family laboratory selection imposed here. This variance contributed to three results. First, the selection restored long 'wild type' or ancestral beak lengths in bugs that had undergone 40–50 years or on the order of 100 generations of selection for short beaks on the introduced goldenrain tree. Second, laboratory selection on the ancestral population produced bugs that had short beaks equivalent in length to the bugs feeding and evolving on goldenrain trees in the field. Third, it was possible to select for even longer beaks in the ancestral population with naturally long beaks and even shorter beaks in the population whose beaks had been selected in that direction in nature. There was thus in both ancestral and derived populations ample genetic variation for reverse evolution and for further forward evolution of either shorter or longer beaks (Fig. 1; Table 2). In these populations of *J. haematoloma* there thus seems to be little if any genetic constraint forestalling evolution in either direction (Gromko, 1995; Teotónio & Rose, 2001). Given the few generations needed for the response in all lines undergoing

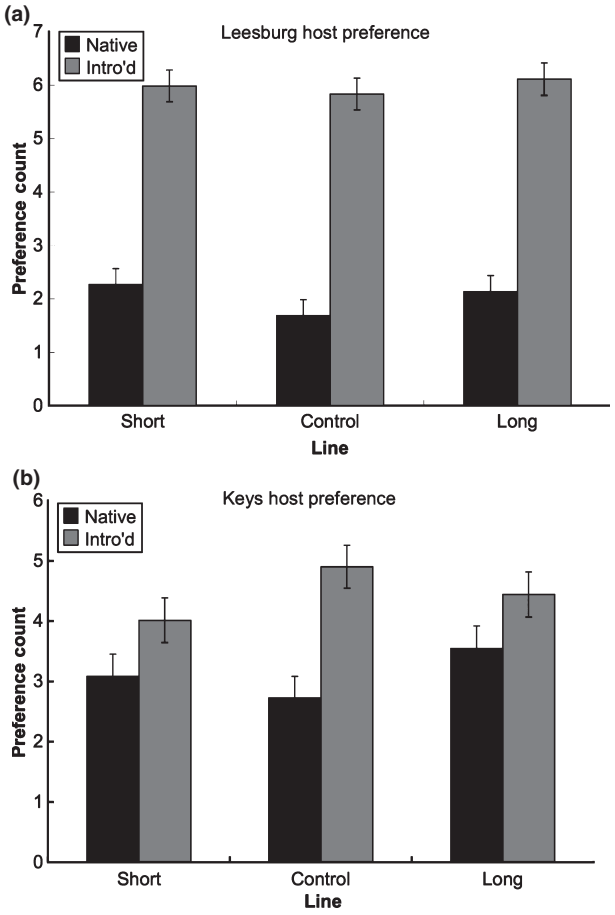

Fig. 4 Host seed preferences in derived Leesburg (a) and ancestral Keys (b) populations after 4–6 generations of directional selection on beak length. Preference count is the mean number of naïve first instar nymphs feeding on a given host seed across all observations (see Methods). The native host is balloon vine (*Cardiospermum*), and the introduced host is goldenrain tree (*Koelreuteria*). Note that both populations preferred the introduced host but that the preference was stronger in the derived Leesburg population naturally occurring on that host.

laboratory selection (Fig. 1), mutation seems an unlikely facilitator of these trends. The most likely facilitator would seem to be sufficient standing genetic variation abetted by pleiotropy (Teotónio & Rose, 2001). Some 100 generations of local adaptation in nature has not altered these factors sufficiently to constrain further evolution in forward or reverse modes.

The genetic correlations between beak length and wing morph frequency observed here (Table 1) suggest that beak length and wing morph are involved in an adaptive suite of traits that is independent of body size (Dingle, 2001). Selection for beak length imposed by the fruit capsule width of the host plant will also select for changes in the frequencies of the alary phenotypes and the flight capability associated with those phenotypes. Examination of the fruiting ecologies of the host plants reveals why a genetic correlation between beak and wing morph frequency may be favoured by selection (Carroll *et al.*, 2003b). The goldenrain tree, on which selection is for short beaks, displays high fruit production with a synchronized seasonal pattern. Selection favours remaining in the vicinity of a single host tree as there is little fitness gain from flying to other trees, which will be in a similar stage of the fruiting cycle. Selection should favour short-beaked bugs with short wings and reduced wing muscles resulting in more reproduction (Carroll & Boyd, 1992; Dingle, 2001). In contrast the balloon vine, on which trophic selection is for long beaks, produces far fewer fruits per plant and does so asynchronously with little reference to season. Selection in this case should favour long-beaked bugs with a functional flight system that can fly readily from hosts with little or declining fruit production to hosts where fruits are in greater abundance. A higher frequency of long-winged morphs is indeed observed in the field in the long-beaked ancestral balloon vine populations.

In other insects that have been studied, suites of traits involved in migration include reproductive capacity. This is true both where migration is determined by enhanced flight in species in which all individuals can fly and migrants display higher reproduction (Palmer & Dingle, 1986, 1989; Dingle *et al.*, 1988), and in wing polymorphic insects like crickets where short-winged or apterous forms show greater reproductive capacity (reviewed in Roff & Fairbairn, 2007). In the latter case there is usually a trade-off between energy and resources devoted to flight as opposed to reproductive output. We have some evidence here of the genetic correlation of reproduction with beak and wing morph. In both ancestral and derived populations egg mass was reduced in the short-beaked lines (Fig. 2). This is consistent with data from field populations where short-beaked bugs produce more but smaller eggs (Carroll *et al.*, 2003a,b). Egg production was higher in ancestral bugs selected for short beaks, but was reduced in both long- and short-beaked lines in the derived bugs (Fig. 3). There is thus some support for the inclusion of reproductive traits in a suite associated with beak length and wing morph, consistent with what would be expected from our field studies and from field and laboratory studies of other insects. Clearly, however, more replicated studies, with more generations of selection and the determination of flight muscle status for each long-winged bug, are needed before firm conclusions can be drawn. In general more work is needed on the degree to which genetic correlations and covariances respond to environmental change (Lynch & Walsh, 1998, p. 647).

There is mounting evidence that the suites of traits included within insect flight polymorphisms are coordinated by juvenile hormone (JH) and its esterase (JHE) (reviewed in Dingle, 2002). In various field crickets higher titres of JH induce short wings, absence of wing muscles, and higher rates of reproduction; the

responsible hormone and esterase titres are responsive to laboratory selection on wing morph (Zera, 2004, 2006; Roff & Fairbairn, 2007). Application of JH analogues to *J. haematoloma* increases the proportion of the short-winged morph in both ancestral and derived populations. The two populations, however, respond differentially to the treatment, implying gene differences in response capability (Dingle & Winchell, 1997). It is thus likely that a JH/JHE system mediates the genetic regulation of beak lengths and the flight polymorphism (Dingle, 2002).

An important conclusion is that during rapid contemporary evolution complex suites of genetically correlated traits can retain the capacity to evolve further or to reverse their evolution. Understanding such contemporary evolution of local adaptation has the potential to influence strategies for dealing with invasive species and preventing undesirable invasive spread, an approach currently being employed with, for example, cane toads in Australia (Phillips *et al.*, 2006). Reciprocally the response of natives to an invader can be assessed and strategies devised to prevent negative impacts as in the response of native red-legged frogs to introduced bullfrogs in California (Kiesecker & Blaustein, 1997). Clearly complex adaptations of all sorts from morphology to life history can evolve rapidly in response to environmental change, and these adaptations can include genetically correlated traits. These conclusions have implications for community ecology (Thompson, 1998). Furthermore, awareness of constraints or lack thereof on such evolution should help to avoid strategic pitfalls in cases where managing change is desirable regardless of whether the strategic aims involve pest management, conservation, or other forms of ecological interaction (Ashley *et al.*, 2003; Carroll, 2007, 2008). Rapid evolutionary responses to the environmental impacts of global phenomena such as climate change (e.g. Visser, 2008) should instill a sense of urgency to develop a comprehensive understanding of the phenomenon.

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References

Ashley, M.V., Willson, M.F., Pergams, O.R.W., O'Dowd, D.J. & Gende, S.M. 2003. Evolutionarily enlightened management. *Biol. Cons.* **111**: 115–123.

Bradshaw, W.E. 1986. Pervasive themes in insect life cycle strategies. In: *The Evolution of Insect Life Cycles* (F. Taylor & R. Karban, eds), pp. 261–275. Springer-Verlag, New York.

Bradshaw, W.E. & Holzapfel, C.M. 2001. Phenotypic evolution and the genetic architecture underlying photoperiodic time measurement. *J. Insect Physiol.* **47**: 809–820.

Bradshaw, W.E., Haggerty, B.P. & Holzapfel, C.M. 2005. Epistasis underlying a fitness trait within a natural population of the pitcher-plant mosquito, *Wyeomyia smithii*. *Genetics* **169**: 485–488.

Carroll, S.P. 2007. Natives adapting to invasive species: ecology, genes, and the sustainability of conservation. *Ecol. Res.* **22**: 892–901.

Carroll, S.P. 2008. Facing change: forms and foundations of contemporary adaptation to biotic invasions. *Mol. Ecol.* **17**: 361–372.

Carroll, S.P. & Boyd, C. 1992. Host race radiation in the soapberry bug: natural history with the history. *Evolution* **46**: 1052–1069.

Carroll, S.P. & Watters, J.W. 2008. Driving phenotypic variability with genetic and environmental heterogeneity: adaptation as a first principle of conservation management. In: *Conservation Biology – Evolution in Action* (S.P. Carroll & C.W. Fox, eds), pp. 181–198. Oxford University Press, Oxford.

Carroll, S.P., Dingle, H. & Klassen, S.P. 1997. Genetic differentiation of fitness-associated traits among rapidly evolving populations of the soapberry bug. *Evolution* **51**: 1182–1188.

Carroll, S.P., Klassen, S.P. & Dingle, H. 1998. Rapidly evolving adaptations in host ecology and nutrition in the soapberry bug. *Evol. Ecol.* **12**: 955–968.

Carroll, S.P., Dingle, H., Famula, T.R. & Fox, C.W. 2001. Genetic architecture of adaptive differentiation in evolving host races of the soapberry bug, *Jadera haematoloma*. *Genetica* **112–113**: 257–272.

Carroll, S.P., Marler, M., Winchell, R. & Dingle, H. 2003a. Evolution of cryptic flight morph and life history differences during host race radiation in the soapberry bug, *Jadera haematoloma* Herrich-Schaeffer (Hemiptera: Rhopalidae). *Ann. Entomol. Soc. Am.* **96**: 135–143.

Carroll, S.P., Dingle, H. & Famula, T.R. 2003b. Rapid appearance of epistasis during adaptive divergence following colonization. *Proc. Biol. Sci.* **270**: S80–S83.

Carroll, S.P., Loye, J.E., Dingle, H., Mathieson, M., Famula, T.R. & Zalucki, M.P. 2005. And the beak shall inherit – evolution in response to invasion. *Ecol. Lett.* **8**: 944–951.

Dingle, H. 2001. The evolution of migratory syndromes in insects. In: *Insect Movement: Mechanisms and Consequences* (I.P. Woiwood, D.R. Reynolds & C.D. Thomas, eds), pp. 159–181. CABI, Wallingford, U.K.

Dingle, H. 2002. Hormonal mediation of insect life histories. In: *Hormones, Brain and Behavior*, Vol. 3, Ch. 41 (D.W. Pfaff, A.M. Etgen, S.E. Fahrbach & R.T. Rubin, eds), pp. 237–279. Academic Press (Elsevier), San Diego, California.

Dingle, H. & Winchell, R. 1997. Juvenile hormone as a mediator of phenotypic plasticity in insect life histories. *Arch. Insect Biochem. Physiol.* **35**: 359–373.

Dingle, H., Evans, K.E. & Palmer, J.O. 1988. Responses to selection among life-history traits in a nonmigratory population of milkweed bugs (*Oncopeltus fasciatus*). *Evolution* **42**: 79–92.

Falconer, D.S. & MacKay, T.F.C. 1996. *Introduction to Quantitative Genetics*, 4th edn. Longman, Essex, U.K.

Felsenstein, J. 2005. Using the quantitative genetic threshold model for inferences between and within species. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **360**: 1427–1434.

Geman, S. & Geman, D. 1984. Stochastic relaxation, Gibbs distributions and Bayesian restoration of images. *IEEE Trans. Pattern Anal. Mach. Intell.* **6**: 721–741.

Geweke, J. 1992. Evaluating the accuracy of sampling-based approaches to calculating posterior moments. In: *Bayesian Statistics 4* (J.M. Bernardo, J.O. Berger, A.P. Dawid & A.F.M. Smith, eds), pp. 169–193. Oxford University Press, Oxford.

Grant, B.S., Owen, D.F. & Clarke, C.A. 1996. Parallel rise and fall of melanic peppered moths in America and Britain. *J. Heredity* **87**: 351–357.

Gromko, M.H. 1995. Unpredictability of correlated response to selection: pleiotropy and sampling interact. *Evolution* **49**: 685–693.

Hairston, N.G., Ellner, S.P., Geber, M.A., Yashida, T. & Fox, J.A. 2005. Rapid evolution and the convergence of ecological and evolutionary time. *Ecol. Lett.* **8**: 1114–1127.

Hendry, A.P. & Kinnison, M.T. 1999. The pace of modern life: measuring rates of contemporary microevolution. *Evolution* **53**: 1637–1653.

Herrel, A., Huyghe, K., Vanhooydonck, B., Backeljau, T., Breugelmans, K., Grbac, I., Van Damme, R. & Irschick, D.J. 2008. Rapid, large-scale evolutionary divergence in morphology and performance associated traits with exploitation of a different dietary resource. *Proc. Nat. Acad. Sci. U.S.A.* **105**: 4792–4795.

Hyndman, R. 1996. Computing and graphing highest density regions. *Am. Stat.* **50**: 120–126.

Hyndman, R. 2008. hdrcde: highest density regions and conditional density estimation. *R Package Version 2.09*. R Foundation for Statistical Computing, Vienna, URL http//www.robhyndman.info/Rlibrary/hdrcde (last accessed 7 August 2009).

Kawecki, T.J. & Ebert, D. 2004. Conceptual issues in local adaptation. *Ecol. Lett.* **7**: 1225–1241.

Kiesecker, J.M. & Blaustein, A.R. 1997. Population differences in responses of red-legged frogs (*Rana aurora*) to introduced bullfrogs. *Ecology* **78**: 1752–1760.

Lambrinos, J.G. 2004. How interactions between ecology and evolution influence contemporary invasion dynamics. *Ecology* **85**: 2061–2070.

Lynch, M. & Walsh, B. 1998. *Genetics and Analysis of Quantitative Traits*. Sinauer, Sunderland, Massachusetts.

Majerus, M.E.N. 1998. *Melanism: Evolution in Action*. Oxford University Press, Oxford.

O'Dowd, D.J., Green, P.T. & Lake, P.S. 2003. Invasional 'meltdown' on an oceanic island. *Ecol. Lett.* **6**: 812–817.

Orr, A.H. 2005. The genetic theory of adaptation: a brief history. *Nat. Rev. Genet.* **6**: 119–127.

Palmer, J.O. & Dingle, H. 1986. Direct and correlated responses to selection among life history traits in milkweed bugs (*Oncopeltus fasciatus*). *Evolution* **40**: 767–777.

Palmer, J.O. & Dingle, H. 1989. Responses to selection on flight behavior in a migratory population of milkweed bugs (*Oncopeltus fasciatus*). *Evolution* **43**: 1805–1808.

Phillips, B.L., Brown, G.P., Webb, J.K. & Shine, R. 2006. Invasion and the evolution of speed in toads. *Nature* **439**: 803.

Plummer, M., Best, N., Cowles, K. & Vines, K. 2007. coda: output analysis and diagnostics for MCMC. *R Package Version 0.13-4*. R Foundation for Statistical Computing, Vienna (last accessed 10 August 2009).

Pulido, F. 2007. The genetics and evolution of avian migration. *Bioscience* **57**: 165–174.

R Development Core Team 2005. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna. URL http://www.R-project.org (last accessed 7 August 2009).

Roff, D.A. & Fairbairn, D.J. 2007. The evolution and genetics of migration in insects. *Bioscience* **57**: 155–164.

Roff, D.A. & Mousseau, T. 2005. The evolution of the phenotypic covariance matrix: evidence for selection and drift in *Melanoplus*. *J. Evol. Biol.* **18**: 1104–1114.

Sax, D.F., Stachowicz, J.J., Brown, J.H., Dawson, M.N., Gaines, S.D., Grosberg, R.K., Hastings, A., Holt, R. D., Mayfield, M.M., O'Conner, M.I. & Rice, W.R. 2007. Ecological and evolutionary insights from species invasions. *Trends Ecol. Evol.* **22**: 465–471.

Siegler, D.S. & Kawahara, W. 1976. New reports of cyanolipids from sapindaceous plants. *Biochem. Syst. Ecol.* **4**: 263–265.

Singer, M.C., Thomas, C.D. & Parmesan, C. 1993. Rapid human-induced evolution of insect-host associations. *Nature* **366**: 681–683.

Smith, B.J. 2007. boa: An R package for MCMC output convergence assessment and posterior inference. *Journal of Statistical Software* **21**: 1–37.

Sorensen, D.A. & Gianola, D. 2002. *Likelihood, Bayesian, and MCMC methods in Quantitative Genetics*. Springer, New York.

Sorensen, D.A., Andersen, S., Gianola, D. & Korsgaard, I. 1995. Bayesian inference in threshold models using Gibbs sampling. *Genet. Sel. Evol.* **27**: 229–249.

Strauss, S.Y., Lau, J.A. & Carroll, S.P. 2006. Evolutionary responses of natives to introduced species: what do introductions tell us about natural communities? *Ecol. Lett.* **9**: 357–374.

Teotónio, H. & Rose, M.R. 2001. Perspective: reverse evolution. *Evolution* **55**: 653–660.

Teotónio, H., Chelo, I.M., Bradić, M., Rose, M.R. & Long, A.D. 2009. Experimental evolution reveals natural selection on standing genetic variation. *Nat. Genet.* **41**: 251–257.

Thompson, J.N. 1998. Rapid evolution as an ecological process. *Trends Ecol. Evol.* **13**: 329–332.

Van Tassel, C.P. & Van Vleck, L.D. 1996. Multiple-trait Gibbs sampler for animal models: flexible programs for Bayesian and likelihood-based (co)variance component inference. *J. Anim. Sci.* **74**: 2586–2597.

Visser, M.E. 2008. Keeping up with a warming world; assessing the rate of adaptation to climate change. *Proc. Biol. Sci.* **275**: 649–659.

Winchell, R., Dingle, H. & Moyes, C.D. 2001. Enzyme profiles in two wing polymorphic soapberry bug populations (*Jadera haematoloma*: Rhopalidae). *J. Insect Physiol.* **46**: 1365–1373.

Zera, A.J. 2004. The endocrine regulation of wing polymorphism in insects: state of the art, recent surprises and future directions. *Integr. Comp. Biol.* **43**: 607–616.

Zera, A.J. 2006. Evolutionary genetics of juvenile hormone and ecdysteroid regulation in Gryllus: a case study in the microevolution of endocrine regulation. *Comp. Biochem. Physiol. Part A Mol. Integr. Physiol.* **144**: 365–379.

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