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Rapid appearance of epistasis during adaptive divergence following colonization

Scott P. Carroll[1*], Hugh Dingle[1] and Thomas R. Famula[2]

[1]*Department of Entomology and Centre for Population Biology, and* [2]*Department of Animal Science, University of California—Davis, Davis, CA 95616, USA*
[*] *Author for correspondence* (*spcarroll@ucdavis.edu*).

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Theory predicts that short-term adaptation within populations depends on additive (A) genetic effects, while gene–gene interactions 'epistasis (E)' are important only in long-term evolution. However, few data exist on the genetic architecture of adaptive variation, and the relative importance of A versus non-additive genetic effects continues to be a central controversy of evolutionary biology after more than 70 years of debate. To examine this issue directly, we conducted hybridization experiments between two populations of wild soapberry bugs that have strongly differentiated in 100 or fewer generations following a host plant shift. Contrary to expectation, we found that between-population E and dominance (D) have appeared quickly in the evolution of new phenotypes. Rather than thousands of generations, adaptive gene differences between populations have evolved in tens. Such complex genetic variation could underlie the seemingly extreme rates of evolution that are increasingly reported in many taxa. In the case of the soapberry bug, extraordinary ecological opportunity, rather than mortality, may have created hard selection for genetic variants. Because ultimate division of populations into genetic species depends on epistatic loss of hybrid compatibility, local adaptation based on E may accelerate macroevolutionary diversification.

Keywords: adaptation; colonization; epistasis; insect; rapid evolution; soapberry bug

1. INTRODUCTION

Epistasis (E), where the expression of alleles depends on the presence of alleles at other loci, is a type of complex genetic architecture underlying variation in many animal and plant characteristics (Wade 2002). Its importance in comparison to non-interactive (additive (A)) genetic effects in development and evolution has been contended since the classic Fisher–Wright debate on the subject emerged in the 1930s (Coyne *et al.* 2000; Goodnight & Wade 2000; Merilä & Sheldon 2000).

Theoretical predictions (Jones 1987) and empirical evidence (Mousseau & Roff 1987) of relatively low A genetic variation for fitness-related traits have heightened focus on sources of variation, including environmental variance (Kruuk *et al.* 2000) and non-additive (E, dominance (D)) genetic variance (Brodie 2000; Merilä & Sheldon 2000; Templeton 2000; Agrawal *et al.* 2001; Wade 2001). In theory, non-additive variance for fitness may persist and increase in relative importance if directional selection removes A variance more quickly than it is generated by mutation (Blows & Hoffman 1996), a process that may plausibly take thousands of generations to occur (Bradshaw & Holzapfel 2000).

Central to the current debate is the importance of E within, versus between populations (Wade 2001; Coyne *et al.* 2000; Goodnight & Wade 2000). Indeed, both D and E fitness differentiation among ecologically distinct populations and species have recently been reported (Armbruster *et al.* 1997; Hatfield 1997). Here, we ask for the first time to our knowledge whether non-additive control plays a part *very early* in the process of adaptive differentiation between populations, perhaps before the loss of substantial A genetic variation.

As an insect currently in the midst of rapid adaptive evolution on recently adopted species of host plants, the soapberry bug, *Jadera haematoloma*, gives a clearer picture of the interaction of genes and environment during population divergence than taxa that diverged in the distant past. This insect is a member of an indigenous New World genus of seed-feeding true bugs. Rapid adaptive evolution has followed the insect's colonization of plant species introduced to North America in the past several decades (Carroll *et al.* 2001). Unchanged populations still persist on the native host species, and so serve as a basis for measuring evolutionary direction and rate in the colonists. In fewer than 100 generations, contrasts in flight and feeding morphology, host preference, development and reproduction have all evolved in response to selection imposed by the new hosts (Carroll *et al.* 2001, 2003).

To examine the quantitative genetic basis of adaptation, we measured three traits from complementary functional classes: length of the mouthparts or 'beak' (functional morphology), host preference (behaviour) and juvenile survivorship (developmental performance). We used joint-scaling analysis (Mather & Jinks 1982) to assess models of A, D, E and maternal effects (M). We used data from the reciprocal backcrosses to estimate the components of digenic E, including $A \times A$, $A \times D$, $D \times D$, $M \times A$ and $M \times D$ effects.

2. METHODS

(a) *Insects*

We collected the grandparental generation of soapberry bugs (*J. haematoloma*) from introduced and native host plants in Florida in 1993. Soapberry bugs (*Jadera* spp.) are specialized seed predators of plants in the family Sapindaceae. In Florida, the native host is the balloon vine (*Cardiospermum corindum*), which occurs at the southern tip of the peninsula and in the Florida Keys. The introduced host is the South East Asian goldenrain tree (*Koelreuteria elegans*), which since *ca.* 1955 has been increasingly planted for landscaping purposes. Precisely how soon after its introduction this plant became an important host is not known. It is most common in central and northern peninsular Florida, such that there is little overlap between the geographical ranges of the two host species. Thus the two host-associated populations in Florida generally occur hundreds of kilometres apart, and no intermediate 'hybrid zone' is known. Two to three generations are produced per year on the introduced host.

(b) *Hybridization experiment*

We began with adults from the ancestral-type race (Plantation Key, 24°70′ N, 80°33′ W) and the derived race (Lake Wales, 27°54′ N, 81°35′ W). Fifty pairs of adult males and females from each site were distributed 10 each into five breeding cages, with water and seeds

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Table 1. Percentage of the total variance explained by the models when fitted to the character means.
(Each of the analyses per trait (by host) includes A as a parameter.)

		percentage variance explained beyond that explained by A alone			
trait host	A	D	D × E	D × M	D × M × E
beak length on:					
native	65	20***	31***	22**	35***
introduced	54	1	43****	23****	46****
juvenile survivorship on:					
native	40	25**	49*	25	53
introduced	37	4	16	14	55
host preference	64	2	25	14	34

*$p \leqslant 0.10$, **$p < 0.05$, ***$p < 0.01$, ****$p < 0.001$.

from the natal host genus. Their virgin adult offspring were removed and paired individually and at random *inter se* (within-line) to produce the parental (P_1) experimental generation. The ensuing lines were established by pairing 20 males and females from the appropriate source(s), fed on the seeds of the female's natal host. Fifty offspring from each of the 80 total families were reared with broods split such that half were on the seeds of one host, and half on the other. We chose resulting adults at random to establish first generation (G_1) pairs. For each of the control lines we established two pairs per family. For each family of the hybrid lines we established two pairs in *inter se* matings, and one pair each of a maternal backcross and a paternal backcross. Second generation (G_2) broods were likewise split between the host, in groups of *ca*. 20 individuals.

(c) *Trait measurements*

Beak length was measured with hand-held digital calipers (0.01 mm measurement interval). Survivorship was measured in groups of *ca*. 20 siblings per rearing box, calculated as the number maturing divided by the number initially inserted as hatchlings. Host preference was measured in separate groups of naive hatchlings from the same families. Because the insects often feed in groups in nature, we tested host preference in groups. Fifteen to twenty hatchlings from each line were placed in clear plastic boxes (18 cm × 12.5 cm × 5 cm high); three fresh seeds of each host were placed at opposite ends of the floor, and the number of individuals feeding on each host was recorded at 3 h intervals over a 9–12 h period each day, until a maximum of 10 records was obtained.

(d) *Data analysis*

Each host preference observation was reduced to a single value, the number of individuals feeding on the native host minus those feeding on the introduced host. Evaluation of these data began with the GLM procedure of SAS (SAS Institute 1996) in a model including terms for 'cross' (e.g. parental lines, F_1, F_2 and subsequent backcrosses) and 'family nested within cross'. The family term was included as a random effect to accommodate the repeated preference observation. The number of individuals per cage was included as an effect in the model. An analysis of residuals from this model using the Wilks' *W* criterion showed that our measure of preference was normally distributed.

We tested for A, D, E and M contributions to divergence of the parental races with joint-scaling tests of phenotypic means, a weighted least-squares multiple regression technique that scales for differences in population means (Lynch & Walsh 1998). However, in the case of food preference, this analysis was built upon the least-squares means (and standard errors of those means) analysis to accommodate the repeated measure of preference. We performed the tests in the order A, AD, ADE, ADM and ADME.

We used a goodness-of-fit test to compare observed to predicted line means (Lynch & Walsh 1998). This statistic is the basis of the calculation of the percentage of the total variance accounted for by each model in table 1, estimated as the coefficient of determination (i.e. r^2). To evaluate whether the addition of each model parameter significantly improved goodness-of-fit, we compared the χ^2-statistic for each model (e.g. AD versus ADE) with the equivalent of a likelihood-ratio test statistic (Lynch & Walsh 1998).

3. RESULTS

The three traits differed in their responses to genetic and environmental manipulation (figure 1*a–c*). Beak length, which has evolved to match the size of host fruit capsules (Carroll *et al.* 2001), was comparatively unaffected by rearing host, especially in the purebred lines, but was strongly influenced by genetic background in a non-additive pattern. Hybrid means differed from the parental mean intermediate values expected from strict A, with large values resulting from E and M effects and their interaction, as well as D effects in those offspring reared on the native, but not on the introduced, host.

Averaged across the two hosts, A effects accounted for *ca*. 60% of the between-population variance, and non-additive effects accounted for the remaining 40% (table 1).

Survivorship, in contrast to beak length, was strongly influenced by host. Figure 1*b* shows a clear pattern of reciprocal interaction. The evolved gain in performance on the introduced host (visualized by comparing the 58% survivorship of the ancestral-type purebred on the introduced host, in contrast to the 82% survivorship of the derived purebred on the same host) is mirrored by a simultaneously evolved loss of performance on the native host (from 84% in the ancestral to 68% in the derived).

Hybrids were intermediate in each rearing host treatment, meaning that any natural hybrid matings would tend to produce fewer offspring than would purebred matings on a local host, thereby reducing any potential gene flow between them. Nonetheless, deviation from stepwise intermediacy resulted from significant D and probably E (table 1). In addition, on the introduced host, the full model (ADME) was significantly more explanatory than ADE ($p < 0.05$), and also differed from AD and ADM (p-values < 0.10).

We studied the third trait, host preference, because of its potential importance in the transition from one host to another as well as to gene flow. Figure 1*c* shows the mean (± 1 s.e.) preference values, measured as feeding frequencies for groups of siblings given a choice between seeds of the two host species. Preference for the introduced host was far greater in the purebred descendants of the derived race than in the ancestral-type race. Genetically intermediate lines tended to be phenotypically intermediate in a pattern proportional to respective contri-

Table 2. Contrast of goodness-of-fit statistics (χ^2-values) for models of A, D, E and M.

		beak length		juvenile survivorship		
model contrast	d.f.	native	introduced	native	introduced	preference
D–A	1	9.67***	2.49	4.42**	0.82	0.50
ADM–AD	2	0.94	32.43****	0.08	1.73	2.40
ADE–AD	3	5.33	60.19****	4.29	2.07	4.51
ADME–ADM	3	6.33*	32.12****	4.83	7.30*	3.92
ADME–ADE	2	1.94	4.37	0.62	6.96**	1.81

*$p \leqslant 0.10$, **$p < 0.05$, *** $p < 0.01$, **** $p < 0.001$.

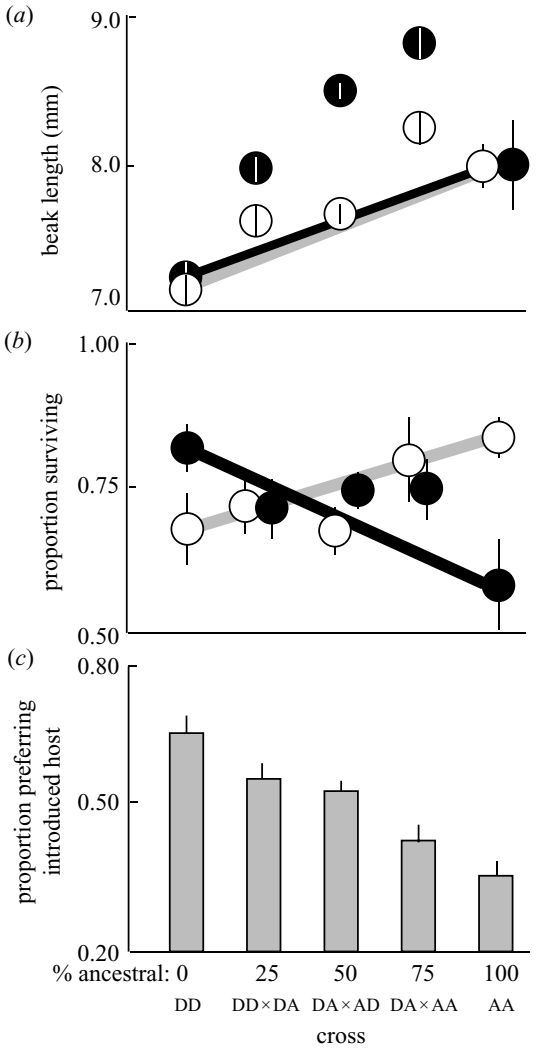

Figure 1. Host influence on the trait depends on the genotype. (*a*) Mean (± 1 s.e.) beak lengths of ancestral-type and derived purebreds and hybrids reared on seed of the native host (white circles) or the introduced host (black circles). (*b*) Mean survivorship to adulthood. Heavy grey or black lines connect the average means for purebreds reared on the respective hosts, providing a reference for visualizing deviations from the additivity hypothesis, which predicts hybrid means on the line. (*c*) Mean hatchling host preference. Abbreviations: AA, the ancestral-type race; DD, the derived race and their combinations; the hybrids and backcrosses. The term '% ancestral' indicates the proportion of the ancestral-type genome present in each cross.

butions of the parental genotypes. Almost two-thirds of phenotypic variation were attributable to A factors, there was no indication of D, and the effects of other non-additive factors, including those of E, were not statistically significant (table 1).

Higher-order contrasts among the models showed that E remained significant in several comparisons (table 2). The addition of E to 'A × D' and to 'A × D × M' substantially improved fit for beak length on the introduced host. Its addition to 'A × D × M' also improved fit for beak length on the native host as well and for survivorship on the introduced host. M similarly enhanced the fit of 'A × D' for beak length on the introduced host, and that of 'A × D × E' for survivorship on that host as well (table 2).

4. DISCUSSION

It is intriguing, if not entirely surprising, to find dissimilar architecture underlying the simultaneous rapid adaptation of different, yet functionally interrelated traits. Combined with complementary results from two related characters—body size and development time—in which D and E, respectively, were more influential than A (Carroll *et al.* 2001), our findings reveal complex genetic organization underlying adaptive population differentiation. Our studies of soapberry bugs accord with those of several species in demonstrating major non-additive genetic influence in the divergence between populations.

What is unexpected in our results, however, *is* the prominence of non-additive control, particularly E, so early in the process of diversification. Previous comparisons revealing non-additive differentiation have examined taxa separated for many hundreds or thousands of generations (e.g. Bradshaw & Holzapfel (2000) and related papers in that volume). Our test indicates that this perspective should be re-evaluated. We believe that the patterns we observed evolved from mass selection on favourable mutations and/or latent extant genetic variation. Whether our evidence of statistical non-additivity reflects the influence of physiological E is uncertain: the presence of any relevant latent variation could have reduced the number of mutations required at interacting loci, if selection acted to rearrange loci already present. The surprising speed and magnitude of soapberry bug adaptive evolution may explain the depth of the genetic differentiation. It is likely that the extraordinary opportunity presented by the novel, unoccupied host, the seed production of which dwarfs that of the native host (Carroll *et al.* 2003), was the 'hard' selection pressure favouring major genetic variants. Thus

we find at present a complex architecture of genetic divergence in traits that to varying degrees are still under the control of quantitative loci.

Theoretical groundwork for examining the population-level consequences of such complex evolution is being advanced (Agrawal *et al.* 2001; Wade 2002). The potential for adaptive epistatic differentiation to produce genetic incompatibility with parental taxa through pleiotropy should be examined as an unanticipated component of swiftly evolving reproductive isolation in vertebrates (e.g. Hendry *et al.* 2001) and in the adaptive radiation of groups such as plant-feeding insects. We are currently investigating this possibility in our study populations.

In summary, we have found that genetic architecture may differentiate under natural selection in tens of years, or 100 generations or fewer. Just as in cases of longer-term evolution, divergence of rapidly evolving populations can result in non-additive genetic differentiation. Rather than being epiphenomenal to evolutionary change, non-additive genetic variation may serve as a foundation for adaptive evolutionary divergence.

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