Genet. Res. Camb. (2002), **80**, *pp.* 215–223. *With* 1 *figure.* © 2002 Cambridge University Press
DOI: 10.1017/S0016672302005876 Printed in the United Kingdom

Variation in *Drosophila* sensory bristle number at 'Evolution Canyon'

RICHARD F. LYMAN[1], EVIATAR NEVO[2] AND TRUDY F. C. MACKAY[1]*
[1] *Department of Genetics, Box 7614, North Carolina State University, Raleigh, NC 27695, USA*
[2] *Institute of Evolution, University of Haifa, Mount Carmel, Haifa 31905, Israel*

(*Received 21 September 2001 and in revised form 31 May 2002*)

Summary

'Evolution Canyon' on Mount Carmel, Israel, displays highly contrasting physical and biotic environments on a micro-geographic scale, and is a natural laboratory for investigating genetic responses to variable and extreme environments across species. Samples of *Drosophila melanogaster* and *D. simulans* were collected from three sites each on the north- and south-facing slopes of the canyon along altitudinal transects, and one site on the valley floor. Numbers of abdominal and sternopleural sensory bristles were recorded for each of these subpopulations in three thermal environments. In *D. simulans*, sternopleural bristle number exhibited micro-geographic differentiation between the north- and south-facing slopes, while abdominal bristle number was stable across subpopulations. In *D. melanogaster*, the magnitudes of the difference in mean sternopleural bristle number between the north- and south-facing slopes and of mean abdominal bristle number along the altitudinal gradients were both conditional on rearing temperature. Thus, the pattern of genetic variation between sites was consistent with underlying heterogeneity of genetic mechanisms for response to the same environmental gradients between traits and sibling species. In contrast, the genetic architecture of bristle number at the level of variation within populations was very similar between species for the same bristle trait, although the two traits differed in the relative contribution of genotype by temperature and genotype by sex interaction.

1. Introduction

A major challenge of evolutionary quantitative genetics is to elucidate the relative contributions of multiple interacting forces on the deposition of genetic variation for quantitative traits within and between natural populations, given spatially and temporally heterogeneous environments. We can postulate various scenarios, depending on the relationship of the trait and the underlying loci affecting variation in the trait to fitness (Robertson, 1967; Falconer & Mackay, 1996). For example, if there is directional or stabilizing selection for an optimal trait value, and the optimum is the same across all environments (populations), selection can reduce genetic variation within populations and favour genotypes that are insensitive to environmental change. If the optimum changes in different environments, selection may also lead to a reduction in genetic variation within populations and favour phenotypically plastic 'generalist' genotypes; alternatively, selection may be for 'specialist' genotypes for each local environment and the evolution of geographic differentiation. Indeed, strong evidence that natural selection has caused genetic differentiation between populations is association of the phenotype with an environmental cline that is replicated across different geographical sampling sites and species (Endler, 1986). Genetic variation for selectively neutral loci will be maintained by mutation–drift balance (Lynch & Hill, 1986) within populations. The extent to which there is geographic differentiation between populations for such loci depends on the migration rate between populations, but in general, no association of mean trait values with clinal variation in environmental parameters is expected.

Determining the interplay between these multiple evolutionary mechanisms in the patterning of genetic

* Corresponding author. Tel: +1 (919) 515 5789. Fax: +1 (919) 515 3355. e-mail: trudy_mackay@ncsu.edu

variation for quantitative traits within and between populations will ultimately be possible only when we know the genetic basis of naturally occurring variation for the traits, at the level of molecular variants defining functional quantitative trait locus (QTL) alleles. While no quantitative trait is currently understood at this level of detail, much progress has been made in mapping QTLs (Long *et al*., 1995; Gurganus *et al*., 1998, 1999; Nuzhdin *et al*., 1999) and identifying corresponding genetic loci and functional alleles (Long *et al*., 1996, 1998, 2000; Gurganus *et al*., 1999; Lyman & Mackay, 1998; Lyman *et al*., 1999) for numbers of sensory bristles in *D. melanogaster*. There is clinal variation for both abdominal and sternopleural bristle number in North America, Africa and Europe, and Australia in both *D. melanogaster* (Lemeunier *et al*., 1986; Coyne & Beecham, 1987; Capy *et al*., 1993) and *D. simulans* (Capy *et al*., 1993), with bristle numbers increasing with latitude. The latitudinal cline parallels the trend for phenotypic plasticity of bristle numbers in response to temperature (Coyne & Beecham, 1987; Gurganus *et al*., 1998) and suggests that temperature may be a major selective agent responsible for the clines.

Evaluation of the role of natural selection in genetic differentiation between populations is facilitated where there are sharp environmental contrasts among populations, as exemplified by Lower Nahal Oren on Mount Carmel, Israel. This canyon exhibits extreme gradients for temperature, insolation and moisture across a narrow geographic range (Nevo, 1995, 1997). The opposite slopes are only 400 m apart at the top and separated by 100 m at the bottom, but the south-facing slope (SFS) is exposed to much higher solar radiation and is warmer, drier, has a more patchy distribution of habitats, and is more heterogeneous spatially and temporally than the relatively homogeneous and temperate north-facing slope (NFS) (Nevo, 1995, 1997). Dubbed 'Evolution Canyon', the opposite slopes have been found to vary for species distribution and richness and genetic diversity in a manner that parallels worldwide geographic trends across a broad range of taxa (Nevo, 1995, 1997). Indeed, adaptative differences between the NFS and SFS of Evolution Canyon have been observed in *Drosophila melanogaster* and *D. simulans* for oviposition preference temperature, viability and longevity under short- and long-term heat stress (Nevo *et al*., 1998); and for positive assortative mating of *D. melanogaster* subpopulations from the same slope (Korol *et al*., 2000; Iliadi *et al*., 2001). Analysis of polymorphism at microsatellite loci shows considerable genetic divergence between *D. melanogaster* populations sampled from the two slopes despite their close proximity, suggesting that selection for habitat choice and assortative mating is a more powerful force than migration at this microsite (Michalak *et al*., 2001).

Because *Drosophila* sensory bristles are excellent traits for addressing fundamental questions in evolutionary quantitative genetics, eventually at the level of individual loci – and there is exceptionally strong divergence in the physical and biotic environments of the opposite slopes of Evolution Canyon – we evaluated the magnitude of genetic variation for abdominal and for sternopleural bristle number within and between sampling sites along environmental gradients at Evolution Canyon in *D. melanogaster* and *D. simulans*, at three developmental temperatures. These data enable us to assess whether there is clinal variation in bristle numbers and genotype by environment interaction for bristle number, and to compare and contrast patterns of genetic variation across bristle traits and species.

2. Materials and methods

(i) Drosophila *stocks*

Isofemale lines of *D. melanogaster* and *D. simulans* were collected from seven sampling sites on Mt Carmel, near Haifa, Israel, in 1994, using fruit-baited traps. Three sampling sites were on the SFS, three on the NFS and one on the canyon floor. The sites on the two slopes were 60 m, 90 m and 120 m above sea level. The lines were maintained in the laboratory as mass mated cultures at 25 °C. It should be noted that one cannot guarantee that the flies collected at each site were residents, and that there was no inbreeding or adaptation to laboratory conditions in the several years the lines were laboratory-reared between the time of collection and the time of evaluation of bristle number. However, collection of non-local females and laboratory adaptation minimize any real differences between populations; and bristle traits do not exhibit inbreeding depression (Falconer & Mackay, 1996).

(ii) *Culture conditions and bristle number phenotypes*

Flies from five isofemale lines of each species from each collection site were reared on standard cornmeal–agar–molasses medium at each of three developmental temperatures: 18, 25 and 28 °C. There were two replicate vials for each isofemale line at each temperature, each seeded with 3–5 mated females and 3–5 males. Abdominal and sternopleural bristle numbers were scored for 10 males and 10 females per replicate vial. Abdominal bristle number was measured as the number of bristles on the most posterior abdominal sternite: segment 6 in females and 5 in males. The total number of sternopleural bristles (macrochaetae and microchaetae) was recorded separately for the right (R) and left (L) sternopleural plates. We computed the total number of sternopleural bristles, L + R; a measure of

fluctuating asymmetry, $|L-R|/(L+R)$; and a measure of directional asymmetry, $(L-R)/(L+R)$. The design was nearly completely balanced.

(iii) *Statistical analyses*

Variance in bristle number was partitioned into sources attributable to temperature (T), collecting site (Si), sex (Se) and species (Sp) by four-way factorial analyses of variance (ANOVA) according to the following mixed model:

$$\begin{aligned} Y = {} & \mu + T + Si + Se + Sp + T \times Si + T \times Se + T \times Sp \\ & + Si \times Se + Si \times Sp + Se \times Sp + T \times Si \times Se \\ & + T \times Si \times Sp + Sp \times Si \times Se + Sp \times T \times Se \\ & + T \times Si \times Se \times Sp + L(Si \times Sp) + T \times L(Si \times Sp) \\ & + S \times L(Si \times Sp) + T \times S \times L(Si \times Sp) \\ & + R(Si \times Sp \times T \times L) \\ & + S \times R(Si \times Sp \times T \times L) + \text{Error} \end{aligned}$$

where T, Si, Se and Sp are fixed cross-classified effects; L and R are random effects of isofemale lines and replicate vial, respectively; and parentheses indicate nested effects.

To assess whether variation in bristle number was associated with patterns of micro-geographical variation associated with sites on the NFS and SFS or with altitude, the following four-way ANOVAs were computed for each species separately:

$$\begin{aligned} Y = {} & \mu + A + E + T + S + A \times E + A \times T + E \times T \\ & + A \times S + E \times S + T \times S + T \times A \times E + A \times E \times S \\ & + T \times A \times S + T \times E \times S + T \times A \times E \times S \\ & + L(A \times E) + T \times L(A \times E) + S \times L(A \times E) \\ & + T \times S \times L(A \times E) + R(A \times E \times T \times L) \\ & + S \times R(A \times E \times T \times L) + \text{Error} \end{aligned}$$

Here A, E, T and S are the fixed cross-classified effects of aspect (NFS vs SFS), elevation above sea level, temperature and sex, respectively. All other terms are as defined above. Note that the data from the collecting site on the valley floor were excluded from these analyses.

Analyses of variance and tests of significance of F-ratios were estimated using type III sums of squares and SAS procedures GLM and VARCOMP (SAS Institute, 1988).

3. Results

Mean abdominal and sternopleural bristle numbers from each of the seven collecting sites in three rearing environments are depicted, separately for *D. melanogaster* and *D. simulans*, in Fig. 1. Analyses of variance of bristle number pooled across temperatures, collection sites, species and sexes are given in Table 1, and ANOVAs pooled over sexes and temperatures for each species separately, which also split the geographical micro-environments into the NFS and SFS and elevation, are given in Table 2.

(i) *Abdominal bristle number*

Abdominal bristle number typically exhibits phenotypic plasticity in response to thermal environments (e.g. Coyne & Beecham, 1987; Gurganus *et al.*, 1998), so it is not surprising that the main effect of temperature, averaged over both species and sexes, was highly significant (Table 1). However, the significant temperature by species interaction indicates that there was a difference between species in sensitivity of abdominal bristle number to temperature, attributable to a change in rank order of bristle number effects and also to a difference in the absolute magnitude of sensitivity between the two species. In *D. melanogaster*, the rank order of bristle number from highest to lowest was $18 > 25 > 28$ °C, while for *D. simulans* it was $18 > 28 > 25$ °C. However, the maximum difference in bristle number between temperatures was 1·6 bristles in *D. melanogaster* and only 0·4 bristle in *D. simulans*.

Although there was no micro-geographical variation in abdominal bristle number averaged over species, sex and temperature, there was geographical variation in plasticity of abdominal bristle number among the sampling sites, as indicated by the significant temperature by site interaction (Table 1). Averaged over species and sexes, this interaction was also due to changes in rank order of bristle number effects in the three temperatures among the different sampling sites and to differences among sites in sensitivity. The maximum difference in bristle number between the highest and lowest temperature varied from 1·6 bristles to 0·3 bristle. The geographical variation in sensitivity was, however, species-specific, as indicated by the highly significant temperature by site by species interaction.

Therefore, we examined the temperature by site interactions within each species, partitioning the variation among sites into variation between slopes, among altitudes, and the slope by altitude interaction (Table 2). None of the site by temperature interaction terms was significant for *D. simulans*. However, in *D. melanogaster* there was substantial and complex variation in sensitivity of abdominal bristle number to temperature associated with micro-environments of the sampling sites. The highly significant elevation by temperature interaction occurred because the variance in sensitivity to temperature was different among altitudes. The difference in bristle number between 18 and 28 °C was 2·3, 0·5 and 1·5 bristles for the 120 m, 60 m and 90 m elevations, respectively. Further, the rank order of the different altitudes with respect to bristle number varied

https://doi.org/10.1017/S0016672302005876 Published online by Cambridge University Press

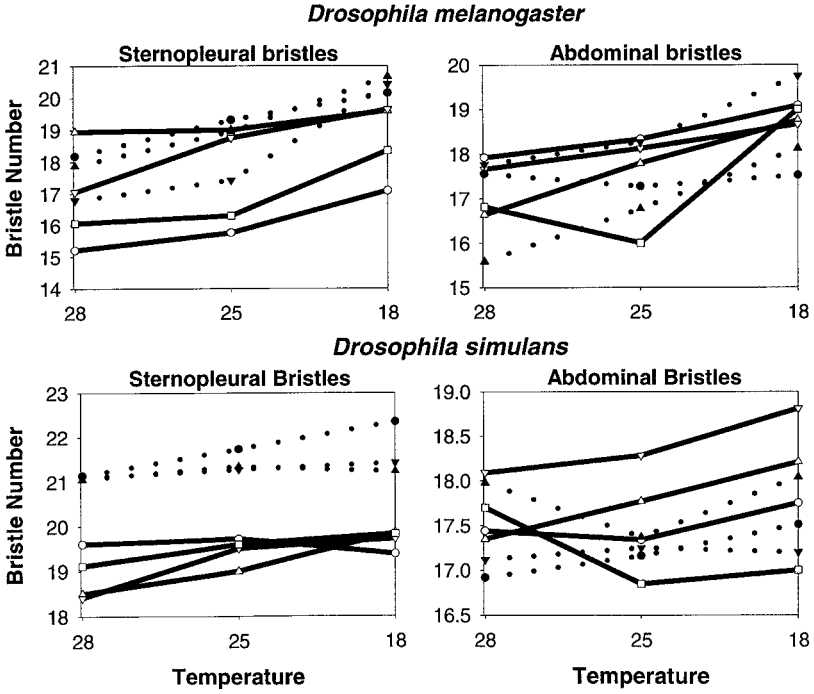

Fig. 1. Mean abdominal and sternopleural bristle numbers of *D. melanogaster* and *D. simulans* from seven collection sites at Evolution Canyon, reared in each of three temperature environments. Continuous lines and open triangles, circles and inverted triangles denote sites from the SFS at 120 mm and 60 m, respectively; while dotted lines and filled triangles, circles and inverted triangles denote sites from the NFS at 120 m, 90 m and 60 m, respectively. The continuous line and open squares represents the site at the canyon floor.

with temperature. At 18 °C, the 60 m sites had on average more abdominal bristles (19·2) than the 90 m and 120 m sites, which were not significantly divergent for bristle number (18·4 and 18·3, respectively). At 28 °C, bristle number was not significantly different between the 60 m and 90 m sites ($\overline{X}=17{\cdot}7$ each), but was very much reduced ($\overline{X}=16{\cdot}1$) at the 120 m sites. Further, temperature-specific effects of elevation on abdominal bristle number also varied between slopes, with much greater temperature-specific variation among altitudes for sites on the NFS than the SFS (Table 2, Fig. 1).

There was sexual dimorphism in bristle number in the whole sample; females had on average 2·2 more abdominal bristles than males. The magnitude of this sexual dimorphism varied between species, and was greater for *D. simulans* than *D. melanogaster*. Further, there was a species-specific difference in the plasticity of sexual dimorphism in bristle number to temperature. The magnitude of the sexual dimorphism effect was stable across all temperatures in *D. simulans*, whereas in *D. melanogaster*, the difference in mean between females and males was 2·1, 1·9 and 1·4 bristles at 18, 28 and 25 °C, respectively.

Within subpopulations, there was highly significant variation in abdominal bristle number and in sexual dimorphism for abdominal bristle number among isofemale lines, for both species. The within-subpopulation genotype by temperature environment interactions were not significant for either species, but in *D. simulans* there was genetic variation in the extent to which sexual dimorphism in bristle number varied with temperature within subpopulations. The relative importance of genotype by sex and genotype by environment interaction is given by $r_{GE}=V_L/(V_L+V_{SL}+V_{TL}+V_{TSL})$, where V_L, V_{SL}, V_{TL} and V_{TSL} are, respectively the among-line, sex by line and temperature by line, and temperature by sex by line variance components from the ANOVAs pooled over sites, environments and sexes (Bulmer, 1985). This ratio was 0·53 in *D. melanogaster* and 0·56 in *D. simulans*, indicating the genetic architecture of abdominal bristle number within subpopulations is similar for these species.

(ii) *Sternopleural bristle number*

There was a significant difference in mean sternopleural bristle number between the two species (18·2 bristles in *D. melanogaster* and 20·2 bristles in *D. simulans*, averaged over sexes, temperatures and collecting sites), and a significant effect of rearing temperature on bristle number (20·0, 19·1 and 18·5 bristles at 18, 25 and 28 °C, respectively, averaged over species, sexes

https://doi.org/10.1017/S0016672302005876 Published online by Cambridge University Press

Table 1. *Analyses of variance of bristle number*

		Abdominal bristles		Sternopleural bristles	
Source	df	MS	*F*	MS	*F*
T	2	692·0	52·0****	1537	69·6****
Si	6	196·0	$1·03^{NS}$	1149	2·55*
Se	1	9465	183****	2713	215****
Sp	1	95·87	$0·506^{NS}$	8616	19·1****
$T \times Si$	12	33·66	2·53**	32·36	$1·46^{NS}$
$T \times Se$	2	16·98	$2·29^{NS}$	2·217	$0·483^{NS}$
$T \times Sp$	2	271·3	20·4****	440·2	19·9****
$Si \times Se$	6	48·83	$0·942^{NS}$	23·99	$1·90^{NS}$
$Si \times Sp$	6	177·8	$0·938^{NS}$	480·6	$1·07^{NS}$
$Se \times Sp$	1	231·5	4·47*	424·0	33·6****
$T \times Si \times Se$	12	5·701	$0·769^{NS}$	3·644	$0·793^{NS}$
$T \times Si \times Sp$	12	50·16	3·77****	53·14	2·40**
$Si \times Se \times Sp$	6	37·29	$0·720^{NS}$	5·611	$0·445^{NS}$
$T \times Se \times Sp$	2	33·74	4·55*	3·236	$0·705^{NS}$
$T \times Si \times Se \times Sp$	12	9·054	$1·22^{NS}$	3·837	$0·835^{NS}$
$L(Si \times Sp)$	55	190·0	3·28****	451·7	15·0****
$T \times L$	110	13·35	$1·20^{NS}$	22·16	2·65****
$Se \times L$	55	51·94	7·00****	12·64	2·75****
$T \times Se \times L$	110	7·425	1·63**	4·599	1·30*
$R(L \times T)$	206	8·242	1·81****	7·307	2·06****
$Se \times R(L \times T)$	206	4·558	$1·03^{NS}$	3·542	1·20*
Error	7425	4·404		2·962	

Sources of variation are temperature (*T*), sampling sites (*Si*), sex (*Se*), species (*Sp*), line (*L*) and replicate vial (*R*).
****$P<0·0001$; ***$0·0001<P<0·001$; **$0·001<P<0·01$; *$0·01<P<0·05$. NS: $P>0·05$.

and sites) (Table 1). As for abdominal bristle number, the plastic response of sternopleural bristle number to temperature was different for the two species. While the rank order of the effects of temperature on bristle number was preserved across species, again *D. simulans* was far less sensitive to temperature than *D. melanogaster*. The average difference in sternopleural bristle number between flies reared at 18 and 28 °C was 0·7 for *D. simulans* and 2·3 for *D. melanogaster*.

Sternopleural bristle number varied among the collecting sites (Table 1). Inspection of the mean values for each site averaged over species, sexes and temperatures indicated that this variation might be clinal, with the three sites from the NFS having a mean of 20·1 and the three sites from the SFS having a mean of 18·6 sternopleural bristles. However, the significant three-way interaction of site, temperature and species (Table 1) indicates that the pattern of geographical variation varied between the species and with temperature. The nature of this complicated interaction is illustrated by the analyses partitioning variation between slopes, altitudes and their interactions, separately for each species (Table 2, Fig. 1).

In *D. simulans*, sternopleural bristle number was indeed significantly greater from sites on the NFS ($\overline{X}=21·4$) than those from the SFS ($\overline{X}=19·3$). The effect of elevation, the slope by elevation interaction, and all site by temperature interactions were not significant for this species.

In contrast, in *D. melanogaster* the main effects of slope, elevation and their interaction were not significant, but all the interactions of these terms with temperature were significant. The dependence of the difference in sternopleural bristle number between the NFS and SFS on temperature was such that there was a large difference in bristle number between the slopes when the flies were reared at 18 °C (1·6 bristles), but little difference at 28 °C (0·5 bristle). Since sternopleural bristle number is always greatest at 18 °C and least at 28 °C, the difference in bristle number between these temperatures can be taken as a measure of the environmental sensitivity, or plasticity, of this trait. Alternatively, then, the slope by temperature interaction can be viewed as greater variation in plasticity for the NFS than the SFS, with a mean plasticity of 2·8 bristles for the NFS and 1·7 bristles for the SFS (Fig. 1). There was also variation in plastic responses to temperature with respect to altitude. The reaction norms of the 90 m and 120 m sites were nearly parallel, with mean plasticities of 1·9 and 1·7 bristles, respectively. However, the mean plasticity for the 60 m sites was 3·8 bristles. The three-way

https://doi.org/10.1017/S0016672302005876 Published online by Cambridge University Press

Table 2. *Analyses of variance of bristle number for each species*

		D. melanogaster					*D. simulans*			
		Abdominal bristles		Sternopleural bristles			Abdominal bristles		Sternopleural bristles	
Source	df	MS	*F*	MS	*F*	df	MS	*F*	MS	*F*
A	1	184	$1{\cdot}26^{NS}$	604	$1{\cdot}49^{NS}$	1	225	$1{\cdot}08^{NS}$	3980	6·45*
E	2	362	$2{\cdot}49^{NS}$	587	$1{\cdot}44^{NS}$	2	76·1	$0{\cdot}367^{NS}$	82·5	$0{\cdot}134^{NS}$
T	2	636	56·0****	1510	74·1****	2	68·6	4·52*	151	6·31**
S	1	3320	78·9****	419	61·0****	1	5770	117****	2380	122****
$A \times E$	2	191	$1{\cdot}31^{NS}$	1030	$2{\cdot}53^{NS}$	2	120	$0{\cdot}577^{NS}$	1·08	$0{\cdot}002^{NS}$
$A \times T$	2	6·26	$0{\cdot}552^{NS}$	105	5·15**	2	11·2	$0{\cdot}741^{NS}$	5·52	$0{\cdot}230^{NS}$
$E \times T$	4	82·2	7·24****	61·3	3·01*	4	2·92	$0{\cdot}193^{NS}$	4·64	$0{\cdot}193^{NS}$
$A \times S$	1	1·08	$0{\cdot}026^{NS}$	19·2	$2{\cdot}79^{NS}$	1	5·52	$0{\cdot}112^{NS}$	12·4	$0{\cdot}634^{NS}$
$E \times S$	2	101	$2{\cdot}41^{NS}$	15·0	$2{\cdot}18^{NS}$	2	24·0	$0{\cdot}489^{NS}$	14·8	$0{\cdot}764^{NS}$
$T \times S$	2	31·2	6·01**	1·56	$0{\cdot}290^{NS}$	2	3·30	$0{\cdot}403^{NS}$	1·33	$0{\cdot}356^{NS}$
$T \times A \times E$	4	31·0	2·73*	64·1	3·15*	4	16·8	$1{\cdot}11^{NS}$	58·9	$2{\cdot}45^{NS}$
$A \times E \times S$	2	59·8	$1{\cdot}42^{NS}$	29·9	4·34*	2	23·2	$0{\cdot}473^{NS}$	12·4	$0{\cdot}638^{NS}$
$T \times A \times S$	2	15·4	$2{\cdot}96^{NS}$	3·69	$0{\cdot}686^{NS}$	2	12·1	$1{\cdot}48^{NS}$	4·81	$1{\cdot}29^{NS}$
$T \times E \times S$	4	4·03	$0{\cdot}776^{NS}$	1·16	$0{\cdot}215^{NS}$	4	9·32	$1{\cdot}14^{NS}$	4·06	$1{\cdot}09^{NS}$
$T \times A \times E \times S$	4	5·24	$1{\cdot}01^{NS}$	6·47	$1{\cdot}20^{NS}$	4	6·04	$0{\cdot}737^{NS}$	0·397	$0{\cdot}107^{NS}$
$L(A \times E)$	23	146	3·02**	407	18·6****	24	207	3·69***	618	15·6****
$T \times L$	46	11·4	$1{\cdot}49^{NS}$	20·3	2·16**	48	15·2	$1{\cdot}23^{NS}$	24·0	3·06****
$S \times L$	23	42·1	8·11****	6·88	$1{\cdot}28^{NS}$	24	49·2	6·00****	19·4	5·21****
$T \times S \times L$	46	5·19	$0{\cdot}916^{NS}$	5·38	1·56*	48	8·19	2·68****	3·73	$1{\cdot}02^{NS}$
$R(T \times L)$	87	8·11	1·43*	7·47	2·17***	90	7·16	2·34****	7·76	2·13***
$S \times R(T \times L)$	87	5·66	1·27*	3·44	$1{\cdot}13^{NS}$	90	3·06	$0{\cdot}769^{NS}$	3·64	$1{\cdot}17^{NS}$
Error	3132	4·44		3·05		3240	3·98		3·10	

Sources of variation are aspect (A), elevation (E), temperature (T), sex (S), line (L) and replicate vial (R).
****$P<0{\cdot}0001$; ***$0{\cdot}0001<P<0{\cdot}001$; **$0{\cdot}001<P<0{\cdot}01$; *$0{\cdot}01<P<0{\cdot}05$. NS: $P>0{\cdot}05$.

interaction between aspect, elevation and temperature was due to a change in the temperature reaction norms of the different elevations between the two slopes. Differences in sternopleural bristle number between 18 °C and 28 °C for the 60, 90 and 120 m sites, respectively, were 3·6, 2·2 and 2·8 bristles for the NFS, and 2·6, 1·9 and 0·6 bristles for the SFS. The shift in the least sensitive altitude between 90 m for the NFS and 120 m for the SFS resulted in a change in rank order and variance among altitudes with temperature between slopes. That is, at 18 °C there was very little difference in bristle number between the 60, 90 and 120 m altitudes on the NFS (20·4, 20·1 and 20·7 bristles, respectively) but on the SFS, the 60 and 120 m altitudes had the same mean bristle number of 19·6, while the 90 m site had a mean of 17·1 bristles. At 28 °C, the rank order of bristle number was 90 m ($\overline{X}=18{\cdot}2$) > 120 m ($\overline{X}=17.9$) > 60 m ($\overline{X}=16{\cdot}8$) on the NFS, but 120 m ($\overline{X}=18{\cdot}9$) > 60 m ($\overline{X}=17{\cdot}1$) > 90 m ($\overline{X}=15{\cdot}2$) on the SFS (Fig. 1).

Sternopleural bristle number is a sexually dimorphic trait; averaged over species, temperatures and sites, females had 1·2 more sternopleural bristles than males. However, the magnitude of the sexual dimorphism is greater for *D. simulans* (1·6 bristles) than for *D. melanogaster* (0·7 bristle) (data not shown). Further, there was a complicated pattern of micro-geographic variation in sexual dimorphism in *D. melanogaster*, as evidenced by the significant aspect by elevation by sex interaction (Table 2). There was variation in the magnitude of the sexual dimorphism of sternopleural bristle number for both slopes, but the rank order of sexual dimorphism among altitudes varied across slopes. On the SFS, the difference between female and male bristle number at 60, 90 and 120 m was 0·9, 0·5 and 0·1, respectively, whereas on the NFS these respective differences were 0·9, 0·4 and 1·2 (data not shown). Unlike abdominal bristle number, the magnitude of the sexual dimorphism for sternopleural bristle number in *D. melanogaster* was not dependent on temperature.

For both species, there was significant segregating genetic variation for sternopleural bristle number within the local subpopulations, as well as genetic variation for temperature plasticity. In *D. simulans*, there was genetic variation for sexual dimorphism in sternopleural bristle number within subpopulations that was sensitive to rearing temperature, whereas in *D. melanogaster*, genetic variation in sexual dimorphism for this trait within subpopulations depended on temperature. Estimates of r_{GE} for sternopleural bristle number were 0·89 for *D. melanogaster* and 0·88 for *D. simulans*, indicating that the general patterning of variation for this trait is similar within local populations of the two species.

https://doi.org/10.1017/S0016672302005876 Published online by Cambridge University Press

We also analysed the sternopleural bristle data for fluctuating asymmetry (FA), thought to be a measure of developmental stability (the absolute value of the difference in bristle number between the right and left sides, scaled by the total bristle number), and directional asymmetry. In the analysis pooled across species, no term was significant for directional asymmetry. However, three terms were significant for FA: the main effect of temperature ($F_{2,110}=5{\cdot}86$, $P=0{\cdot}004$), the temperature by sex interaction ($F_{2,110}=5{\cdot}07$, $P=0{\cdot}008$) and the species by site interaction ($F_{6,95}=2{\cdot}30$, $P<0{\cdot}05$). The temperature effect accrues because the mean FA is less at 18 °C ($\overline{X}=4{\cdot}9\times10^{-2}$) than at 25 °C ($\overline{X}=5{\cdot}3\times10^{-2}$) or 28 °C ($\overline{X}=5{\cdot}4\times10^{-2}$), and the temperature by sex effect because the direction of sex dimorphism for FA changes with temperature. FA is greater for females than males at 25 °C, but greater for males than females at 18 and 28 °C (data not shown). Somewhat intriguingly, micro-geographic variation in mean FA appears to be negatively correlated between the two species ($r=-0{\cdot}75$, $t=2{\cdot}53$, $P<0{\cdot}06$).

4. Discussion

Although *D. melanogaster* and *D. simulans* are closely related sibling species, the populations inhabiting Evolution Canyon are quite divergent for temperature plasticity and sexual dimorphism of bristle number, and the pattern and magnitude of micro-geographic variation in bristle number across the environmental gradients. Both bristle traits are less plastic in *D. simulans* than in *D. melanogaster* across the range of temperatures tested, consistent with previous observations that *D. melanogaster* is more tolerant to temperature and other environmental stresses (David *et al.*, 1983). There is less geographic differentiation in bristle number for *D. simulans* than *D. melanogaster*: the proportion of the total variation explained by the variation among sites, averaged over temperatures, was 2·9 % for abdominal and 12·8 % for sternopleural bristles in *D. simulans*, but 7·4 % for abdominal and 19·7 % for sternopleural bristles in *D. melanogaster*. This is also consistent with previous observations that the geographic range of variation for bristles and other quantitative traits worldwide is much less for *D. simulans* than for *D. melanogaster* (Singh, 1989; Capy *et al.*, 1993). However, the degree and pattern of genetic differentiation between sites is sensitive to rearing temperature in *D. melanogaster*, but not in *D. simulans*. The magnitude of the sexual dimorphism for both bristle traits is greater in *D. simulans* than *D. melanogaster*, but in *D. melanogaster* sexual dimorphism for abdominal bristle number is highly dependent on temperature, and sexual dimorphism for sternopleural bristle number varies geographically.

Overall, the picture above the level of the local population is that *D. melanogaster* is much more plastic than *D. simulans* and exhibits between-site genetic variation in plasticity and sexual dimorphism, whereas *D. simulans* does not. These differences between species disappear at the level of genetic variation within subpopulations, where the genetic architecture of the same bristle trait is very similar for both species in terms of magnitude of variation among lines (as observed by Capy *et al.*, 1994) and for genotype by temperature environment and genotype by sex interactions. However, the two bristle traits vary significantly in this regard, with a much higher proportion of genetic variation for abdominal bristle number than for sternopleural bristle number tied up in interactions with sex and temperature. This appears to be a general phenomenon, and has been observed previously for spontaneous (Mackay & Lyman, 1998) and *P*-element-induced mutations (Lyman *et al.*, 1996) as well as for naturally occurring variation (Lyman & Mackay, 1998) for bristle number in *D. melanogaster*.

Worldwide, there are strong replicated latitudinal clines for both sternopleural and abdominal bristle number, in both *D. melanogaster* and *D. simulans* (Lemeunier *et al.*, 1986; Coyne & Beecham, 1987; Capy *et al.*, 1993). This trend is not exactly recapitulated at Evolution Canyon; we observe variation between species and between traits in the patterning of genetic variation across environmental gradients. The pattern of genetic variation between sites is consistent with different relationships of the bristle traits to fitness in the sibling species.

In *D. simulans*, sternopleural bristle number exhibits micro-geographic differentiation for specialist genotypes between the NFS (cooler) and SFS (warmer), such that bristle number is higher on the NFS, paralleling the latitudinal clines and the direct effect of temperature on bristle number. In contrast, abdominal bristle number is not genetically differentiated among subpopulations in this species, which is consistent with either selective neutrality or the evolution of genotypes that are relatively insensitive to environmental variation.

In *D. melanogaster*, there is geographic differentiation among subpopulations for both bristle traits, but the magnitude and pattern are conditional on rearing temperature. In this species, sternopleural bristle number of flies collected from the NFS is higher than those collected from the SFS when the animals are reared at low temperature, but not when they are reared at high temperatures. Further, sternopleural bristle number is less plastic with temperature in the more variable and more extreme environments. Sites from the SFS are less plastic to temperature than those from the NFS, as are sites from the highest altitudes. The 120 m site from the SFS is the least plastic of all. These observations are consistent with

the evolution of genotypes that are insensitive to environmental variation. A similar reduction in plasticity for the SFS sites has been observed for viability, adult longevity and desiccation resistance under thermal and drought stress (Nevo *et al*., 1998). A different pattern pertains to abdominal bristle number. For this trait, there is no differentiation between flies collected from the opposite slopes, or for slope by temperature interaction. However, abdominal bristle numbers from sites at the highest elevations are considerably more plastic to temperature than the other sites. The direction of the effect is the same as the latitudinal variation and the direct effect of temperature on bristle number, i.e. lower numbers of bristles at high temperature. The three-way interaction between slope, altitude and temperature for this trait is because the highest elevations are most sensitive to temperature for both slopes, but the sites from the 60 m and 90 m elevations are much more stable with temperature on the SFS than the NFS.

These observations strongly suggest the action of natural selection in patterning geographic variation in bristle number across Evolution Canyon, and implicate temperature as one of the selective agents. However, the observations do not implicate bristle numbers as the direct targets of selection, since many other quantitative traits, including body size, also co-vary clinally both on a worldwide scale (Coyne & Beecham, 1987; Capy *et al*., 1993; James *et al*., 1995, 1997) and across these sites (Nevo *et al*., 1998). Identifying the direct target of selection is a non-trivial task. The minimum requirement is to demonstrate that the relationship of the selected trait to fitness varies with temperature in the predicted direction (e.g. McCabe & Partridge, 1997; Reeve *et al*., 2000). However, the relationship of a trait to fitness is not informative as to whether selection is directly on the observed trait or on another trait pleiotropically connected to it (Nuzhdin *et al*., 1995). For example, stabilizing selection on sternopleural bristle number in the laboratory acts on larvae, perhaps through larval competition (Kearsey & Barnes, 1970; Linney *et al*., 1971). Several QTLs affecting naturally occurring variation in bristle number have been identified genetically, and correspond to genes affecting peripheral nervous system development (Long *et al*., 1996, 1998, 2000; Mackay & Lyman, 1998; Gurganus *et al*., 1999; Lyman *et al*., 1999). These genes have pleiotropic effects on the development of the central nervous system, sex determination, embryonic pattern formation, and eye and wing development (Lindsley & Zimm, 1992). Fortunately, the problem of identifying the trait(s) targeted by selection reduces to determining the causal relationship of the underlying loci affecting variation in bristle number to fitness (Robertson, 1967). Since fitness effects at individual QTL are likely to be weak (Kimura, 1983; Kingsolver *et al*., 2001) and one needs to consider all ecologically relevant environments in nature, this question will best be addressed by molecular population genetic analyses of historical selection acting on DNA sequences of cloned QTLs (Kreitman, 1991; Hartl & Clark, 1997; Wang *et al*., 1999).

We thank J. Brant Hackett for technical assistance and Robert Anholt for comments on the manuscript. This work was funded by NIH grant GM 45146 to T.F.C.M., and grants from the Ancell Teicher Research Foundation for Genetics and Molecular Evolution and the Israeli National Science Foundation to E.N. This is a publication of the W. M. Keck Center for Behavioral Biology.

References

Bulmer, M. G. (1985). *The Mathematical Theory of Quantitative Genetics*. Oxford: Clarendon Press.

Capy, P., Pla, E. & David, J. R. (1993). Phenotypic and genetic variability of morphometrical traits in natural populations of *Drosophila melanogaster* and *D. simulans*. I. Geographic variations. *Genetics, Selection, Evolution* **25**, 517–536.

Capy, P., Pla, E. & David, J. R. (1994). Phenotypic and genetic variability of morphometrical traits in natural populations of *Drosophila melanogaster* and *D. simulans*. II. Within-population variability. *Genetics, Selection, Evolution* **26**, 15–28.

Coyne, J. A. & Beecham, E. (1987). Heritability of two morphological characters within and among natural populations of *Drosophila melanogaster*. *Genetics* **117**, 727–787.

David, J. R., Allemand, R., van Herrewege, J. & Cohet, Y. (1983). Ecophysiology: abiotic factors. In *The Genetics and Biology of Drosophila*, vol. 3d (ed. M. Ashburner, H. L. Carson & J. N. Thompson, Jr.), pp. 105–170. London: Academic Press.

Endler, J. A. (1986). *Natural Selection in the Wild*. Princeton, NJ: Princeton University Press.

Falconer, D. S. & Mackay, T. F. C. (1996). *Introduction to Quantitative Genetics*, 4th edn. Harlow, Essex: Addison-Wesley Longman.

Gurganus, M. C., Fry, J. D., Nuzhdin, S. V., Pasyukova, E. G., Lyman, R. F., *et al*. (1998). Genotype–environment interaction for quantitative trait loci affecting sensory bristle number in *Drosophila melanogaster*. *Genetics* **149**, 1883–1898.

Gurganus, M. C., Nuzhdin, S. V., Leips, J. W. & Mackay, T. F. C. (1999). High-resolution mapping of quantitative trait loci for sternopleural bristle number in *Drosophila melanogaster*. *Genetics* **152**, 1585–1604.

Hartl, D. L. & Clark, A. G. (1997). *Principles of Population Genetics*, 3rd edn. Sunderland, MA: Sinauer.

Iliadi, K., Iliadi, N., Rashkovetsky, E., Minkov, I., Nevo, E., *et al*. (2001). Sexual and reproductive behaviour of *Drosophila melanogaster* from a microclimatically interslope differentiated population of 'Evolution Canyon' (Mount Carmel, Israel). *Proceedings of the Royal Society of London, Series B* **268**, 2365–2374.

James, A. C., Azevedo, R. & Partridge, L. (1995). Cellular basis and developmental timing in a size cline of *Drosophila melanogaster*. *Genetics* **140**, 659–666.

James, A. C., Azevedo, R. & Partridge, L. (1997). Genetic and environmental responses to temperature of *Drosophila melanogaster* from a latitudinal cline. *Genetics* **146**, 881–890.

Kearsey, M. J. & Barnes, B. W. (1970). Variation for metrical characters in *Drosophila* populations. II. Natural selection. *Heredity* **25**, 11–21.

Kimura, M. (1983). *The Neutral Theory of Molecular Evolution*. Cambridge: Cambridge University Press.

Kingsolver, J. G., Hoekstra, H. E., Hoekstra, J. M., Berrigan, D., Vignieri, S. N., *et al*. (2001). The strength of phenotypic selection in natural populations. *American Naturalist* **157**, 245–261.

Kondrashov, A. S. & Turelli, M. (1992). Deleterious mutations, apparent stabilizing selection and the maintenance of quantitative variation. *Genetics* **132**, 603–618.

Korol, A., Rashkovetsky, E., Iliadi, K., Michalak, P., Ronin, Y., *et al*. (2000). Nonrandom mating in *Drosophila melanogaster* laboratory populations derived from closely adjacent ecologically contrasting slopes at 'Evolution Canyon'. *Proceedings of the National Academy of Sciences of the USA* **97**, 12637–12642.

Kreitman, M. (1991). Detecting selection at the level of DNA. In *Evolution at the Molecular Level* (ed. R. K. Selander, A. G. Clark & T. S. Whittam), pp. 204–221. Sunderland, MA: Sinauer.

Lemeunier, F., David, J. R., Tsacas, L. & Ashburner, M. (1986). The *melanogaster* species group. In *The Genetics and Biology of Drosophila*, vol. 3e (ed. M. Ashburner, H. L. Carson & J. N. Thompson, Jr.), pp. 147–256. London: Academic Press.

Lindsley, D. L. & Zimm, G. G. (1992). *The Genome of* Drosophila melanogaster. San Diego: Academic Press.

Linney, R., Barnes, B. W. & Kearsey, M. J. (1971). Variation for metrical characters in *Drosophila* populations. III. The nature of selection. *Heredity* **27**, 163–174.

Long, A. D., Mullaney, S. L., Reid, L. A., Fry, J. D., Langley, C. H., *et al*. (1995). High resolution mapping of genetic factors affecting abdominal bristle number in *Drosophila melanogaster*. *Genetics* **139**, 1273–1291.

Long, A. D., Mullaney, S. L., Mackay, T. F. C. & Langley, C. H. (1996). Genetic interactions between naturally occurring alleles at quantitative trait loci and mutant alleles at candidate loci affecting bristle number in *Drosophila melanogaster*. *Genetics* **144**, 1497–1518.

Long, A. D., Lyman, R. F., Langley, C. H. & Mackay, T. F. C. (1998). Two sites in the *Delta* gene region contribute to naturally occurring variation in bristle number in *Drosophila melanogaster*. *Genetics* **149**, 999–1017.

Long, A. D., Lyman, R. F., Morgan, A. H., Langley, C. H. & Mackay, T. F. C. (2000). Both naturally occurring insertions of transposable elements and intermediate frequency polymorphisms at the *achaete-scute* complex are associated with variation in bristle number in *Drosophila melanogaster*. *Genetics* **154**, 1255–1269.

Lyman, R. F. & Mackay, T. F. C. (1998). Candidate quantitative trait loci and naturally occurring phenotype variation for bristle number in *Drosophila melanogaster*: the *Delta-Hairless* gene region. *Genetics* **149**, 993–998.

Lyman, R. F., Lawrence, F., Nuzhdin, S. V. & Mackay, T. F. C. (1996). Effects of single *P* element insertions on bristle number and viability in *Drosophila melanogaster*. *Genetics* **143**, 277–292.

Lyman, R. F., Lai, C. & Mackay, T. F. C. (1999). Linkage disequilibrium mapping of molecular polymorphisms at the *scabrous* locus associated with naturally occurring variation in bristle number in *Drosophila melanogaster*. *Genetical Research* **74**, 303–311.

Lynch, M. & Hill, W. G. (1986). Phenotypic evolution by neutral mutation. *Evolution* **40**, 915–935.

Mackay, T. F. C. & Lyman, R. F. (1998). Polygenic mutation in *Drosophila melanogaster*: gentoype × environment interaction for spontaneous mutations affecting bristle number. *Genetica* **102/103**, 199–215.

McCabe, J. & Partridge, L. (1997). An interaction between environmental temperature and genetic variation for body size for the fitness of adult female *Drosophila melanogaster*. *Evolution* **51**, 1164–1174.

Michalak, P., Minkov, I., Helin, A., Lerman, D. N., Bettencourt, B. R., *et al*. (2001). Genetic evidence for adaptation-driven incipient speciation of *Drosophila melanogaster* along a microclimatic contrast in 'Evolution Canyon', Israel. *Proceedings of the National Academy of Sciences of the USA* **98**, 13195–13200.

Nevo, E. (1995). Asian, African and European biota meet at 'Evolution Canyon' Israel: local tests of global biodiversity and genetic diversity patterns. *Proceedings of the Royal Society of London, Series B* **262**, 149–155.

Nevo, E. (1997). Evolution in action across phylogeny caused by microclimactic stresses at 'Evolution Canyon'. *Theoretical Population Biology* **52**, 231–243.

Nevo, E., Rashkovetsky, E., Pavlicek, T. & Korol, A. (1998). A complex adaptive syndrome in *Drosophila* caused by microclimactic contrasts. *Heredity* **80**, 9–16.

Nuzhdin, S. V., Fry, J. D. & Mackay, T. F. C. (1995). Polygenic mutation in *Drosophila melanogaster*: the causal relationship of bristle number to fitness. *Genetics* **139**, 861–872.

Nuzhdin, S. V., Dilda, C. L. & Mackay, T. F. C. (1999). The genetic architecture of selection response: inferences from fine-scale mapping of bristle number quantitative trait loci in *Drosophila melanogaster*. *Genetics* **153**, 1317–1331.

Reeve, M. W., Fowler, K. & Partridge, L. (2000). Increased body size confers greater fitness at lower experimental temperature in male *Drosophila melanogaster*. *Journal of Evolutionary Biology* **13**, 836–844.

Robertson A. (1967). The nature of quantitative genetic variation. In *Heritage from Mendel* (ed. A. Brink), pp. 265–280. Madison, WI: University of Wisconsin Press.

SAS Institute. (1988). *SAS/STAT User's Guide*, release 6.03. Cary, NC: SAS Institute.

Singh, R. S. (1989). Population genetics and evolution of species related to *Drosophila melanogaster*. *Annual Reviews of Genetics* **23**, 425–453.

Wang, R.-L., Stec, A., Hey, J., Lukens, L. & Doebley, J. (1999). The limits of selection during maize domestication. *Nature* **398**, 236–239.