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Growth temperature and genetic variability of wing dimensions in *Drosophila*: opposite trends in two sibling species

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Summary

Thirteen linear wing dimensions were measured in 10 isofemale lines of *Drosophila melanogaster* and *D. simulans* grown at seven constant temperatures from 12 to 31 °C. Within-line (environmental) variability, estimated by the within-line coefficient of variation (CVw), exhibited similar variation patterns in the two species, that is higher values at extreme (low or high) temperatures. The magnitude of variation was, however, greater in *D. simulans*, which appears to be more responsive to thermal change. A clear hyperbolic relationship between trait mean value and CVw was also observed in both species, arising from measurement errors which are relatively more pronounced on shorter traits. Genetic variability was analysed by considering both the genetic CV (CV_g, evolvability) and isofemale line heritability (intraclass correlation). Both parameters provided independent information, as shown by a lack of correlation between them. Moreover, CVg was negatively correlated with trait mean value, while heritability showed a positive correlation. With respect to thermal environment, both parameters exhibited similar reaction patterns which contrasted the two species. Genetic variability in *D. melanogaster* followed a convex reaction norm, with higher values at extreme (high or low) temperatures, and this observation agrees with previous independent investigations. Surprisingly, *D. simulans* revealed an opposite pattern, with a maximum genetic variability in the middle of the range. Such data point to the danger of drawing general conclusions from the analysis of a single species.

1. Introduction

Genetic variability is essential for the process of adaptation since a higher level of genetic variation will provide a broader basis for selection to act upon. In quantitative traits, the phenotypic variation comprises components due to genetic and environmental causes so that the total variance of a trait can be partitioned into a genetic and an environmental component (Falconer & Mackay, 1996). If the relative amount of genetic variation, usually estimated as heritability, increases, this can lead to a faster response to selection and accelerate the adaptation process.

During the last decade, it has been repeatedly suggested (Hoffmann & Parsons, 1991; Noach *et al.*, 1996; Bijlsma & Loeschcke, 1997; Moeller & Swaddle, 1997) that natural selection is likely to act most of the time under environmental conditions frequently encountered and close to the organism's optimum. Such persistent selection should decrease the genetic variance for fitness-related traits (Fisher, 1930). Non-optimal and stressful environments, on the other hand, are rarely encountered, leaving little opportunity for natural selection. If there is a strong genotype–environment interaction or if, in other words, the correlation among environments is not very high, we might expect an increased heritability in such extreme but unusual environments. A greater heritability under stressing conditions would be beneficial by permitting a more efficient adaptation when the environment is modified and becomes more stressful.

Temperature is certainly a major abiotic factor, especially for ectothermic organisms, offering both optimum conditions and stressful conditions at ex-

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treme low or high temperatures (Precht *et al.*, 1973; Cossins & Bowler, 1987). It is also clear that what is an optimum for one species will be a major stress for another. For example the Antarctic fly *Anatalanta* exhibits an optimum around 5 °C while 12 °C is a heat stress (Vernon & Vannier, 1996). For *Drosophila melanogaster* 12 °C is a cold stress (David *et al.*, 1983, 1997). Within the drosophilid family, a diversity of species are found, with different thermal ranges and optima (Moreteau *et al.*, 1997, Karan *et al.*, 1998, 1999*a*). Temperature has been used as a stress factor in several cases for monitoring variation of heritability of fitness-related traits. Several studies have shown an increase in genetic variation under stress but not for all traits investigated (Murphy *et al.*, 1983; David *et al.*, 1994; Barker & Krebs, 1995; Noach *et al.*, 1996; Imasheva *et al.*, 1998; Karan *et al.*, 1999*b*). Still other authors did not find a consistent increase in genetic variation under stress in any traits they examined (Sgro & Hoffmann, 1998). For a better understanding of this general problem, there is a need for investigating more numerous traits and comparing a diversity of species.

In the present paper, we describe an investigation on 10 isofemale lines of the two sibling species *D. melanogaster* and *D. simulans*. These lines were grown at various constant temperatures (range 12–31 °C) and 13 wing dimensions were measured. The genetic architecture of these traits has been analysed and the main conclusion is that significant variations have been observed according to growth temperature but in opposite directions in the two species. The shapes of the response curves (reaction norms) of each trait were analysed in a previous publication on the same data set (Moreteau *et al.*, 1998); mean values of the traits can be found in that paper.

2. Materials and methods

Wild-living sympatric females of *D. melanogaster* and *D. simulans* were collected in autumn 1992 in a vineyard at Pont de la Maye near Bordeaux (southern France) on banana traps, and isofemale lines of the two species were started. The lines were kept in the laboratory at a temperature of 19 ± 1 °C for 4 months (about 7 generations) prior to the beginning of the experiment. Ten lines were randomly taken and analysed for each species. For each line, 10 males and 10 females were used as parents and transferred to fresh culture vials twice a day at 21 °C. Culture vials were then put in constant-temperature incubators until adult emergence. Larval density was not precisely controlled, and with this procedure ranged between 100 and 200 individuals per vial. This did not affect the results since we used a high-nutrient, killed yeast medium (David & Clavel, 1965) which is very insensitive to crowding effects (for details see Karan *et al.*, 1999*b*). Seven experimental temperatures covering the whole thermal range of the species (12, 14, 17, 21, 25, 28 and 31 °C) were used. Of these temperatures, two extreme ones (12 and 31 °C) are clearly stressful, and 14 and 28 °C may be considered as mildly stressful (David *et al.*, 1983, 1997; Zhivotovsky *et al.*, 1996). After emergence, adult flies were aged for a few days; next, their left wing was removed and mounted on a microscope slide. Ten females were analysed for each line and each temperature. On each wing, 13 different linear measurements were taken with an ocular micrometer, as indicated in Fig. 1. Micrometer units were transformed into mm $\times$ 100 prior to calculations.

Data were analysed with Statistica software (1999). Isofemale line heritabilities were calculated as coefficients of intraclass correlation in appropriate one-way ANOVAs (Hoffmann & Parsons, 1988; Capy *et al.*, 1994). Genetic variance was estimated as $V_B - V_W/n$, where V_B is the between-line variance, V_W is the within-line variance and n the number of individuals per family. Evolvability was estimated as the genetic coefficient of variation, i.e. the ratio of the genetic standard deviation to the mean (Houle, 1992).

3. Results

(i) *Within-line variability*

Within-line variability among full sibs reared in the same environment harbours a major environmental component, and the corresponding variances are given in Table 1. Huge differences were found between traits (range 1·77–33·1 in *D. melanogaster* and 0·91–28·5 in *D. simulans*) which were highly correlated with trait value (compare with Fig. 1). To avoid this scaling effect, we considered the coefficients of variation (CV) which were submitted to a three-way mixed model ANOVA (Table 2). There was no significant difference between species, but significant effects due to trait, temperature and to the species–trait and species–temperature interactions. Surprisingly, in spite of the utilization of a relative measurement, 86 % of the total variability was still due to traits.

We further investigated this phenomenon and found that CVs were strongly and negatively correlated with trait mean value (Fig. 2). The explanation seems to reside in measurement errors. For each linear measure x_i, the non-genetic variability, generally described by e, is in fact the sum of two independent components, according to the following equation:

$$x_i = x + g + e_i + e_m,$$

where x is the mean value of the trait, g a genetic deviation, e_i is an individual non-genetic component related to developmental fluctuations, and e_m the

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Table 1. *Within-line variance of 13 wing traits in* D. melanogaster *and* D. simulans *at different growth temperatures* (*each value has 90 degrees of freedom*)

Trait	12 °C	14 °C	17 °C	21 °C	25 °C	28 °C	31 °C	Mean	SE
D. melanogaster									
1	26·35	25·32	28·34	19·94	19·63	15·42	19·56	22·08	1·75
2	41·51	33·17	35·39	41·59	26·31	23·10	30·46	33·08	2·67
3	5·38	4·77	5·16	4·99	5·18	6·54	6·46	5·50	0·26
4	8·75	6·72	8·30	4·75	6·12	5·13	6·31	6·58	0·56
5	13·42	19·40	11·41	15·29	13·50	8·17	8·25	12·78	1·50
6	13·14	15·45	14·40	16·20	11·35	9·46	9·26	12·75	1·05
7	14·56	11·83	14·10	15·83	12·15	12·30	12·74	13·36	0·56
8	12·03	17·43	13·08	12·42	10·92	9·56	11·79	12·46	0·93
9	18·93	16·50	19·85	14·30	11·06	8·08	11·08	14·26	1·66
10	1·50	2·18	2·09	1·57	1·76	1·39	1·93	1·77	0·11
11	4·68	2·67	2·55	2·44	1·52	1·40	1·59	2·41	0·42
12	7·18	9·44	7·23	9·20	4·69	4·94	5·07	6·82	0·75
13	4·53	6·31	3·49	4·09	3·36	2·89	2·79	3·92	0·46
Mean	13·23	13·17	12·72	12·51	9·81	8·34	9·80		
SE	3·01	2·57	2·80	2·95	2·01	1·68	2·21		
D. simulans									
1	30·73	20·35	14·71	13·81	11·52	13·00	19·58	17·67	2·51
2	47·93	26·36	26·48	24·18	15·14	26·87	32·50	28·50	3·79
3	9·26	12·39	4·79	5·70	5·70	5·12	7·00	7·14	1·04
4	4·93	4·90	3·56	5·41	4·32	2·81	4·74	4·38	0·34
5	19·41	9·90	9·22	6·06	5·45	5·88	8·07	9·14	1·83
6	12·29	7·72	8·41	5·71	4·28	6·25	7·36	7·43	0·96
7	15·24	11·58	9·01	11·44	7·75	10·46	13·21	11·24	0·95
8	14·23	12·47	8·69	7·52	6·21	6·05	11·06	9·46	1·20
9	15·56	9·49	7·83	7·28	4·81	6·75	8·00	8·53	1·29
10	0·81	1·37	0·84	0·95	0·93	0·75	0·74	0·91	0·08
11	2·99	3·26	2·16	2·24	1·29	1·69	1·49	2·16	0·28
12	6·27	5·35	3·89	4·72	2·57	4·22	2·56	4·22	0·52
13	4·49	5·38	3·24	2·32	1·84	1·51	3·16	3·14	0·53
Mean	14·17	10·04	7·91	7·49	5·52	7·03	9·19		
SE	3·60	1·93	1·87	1·70	1·13	1·91	2·42		

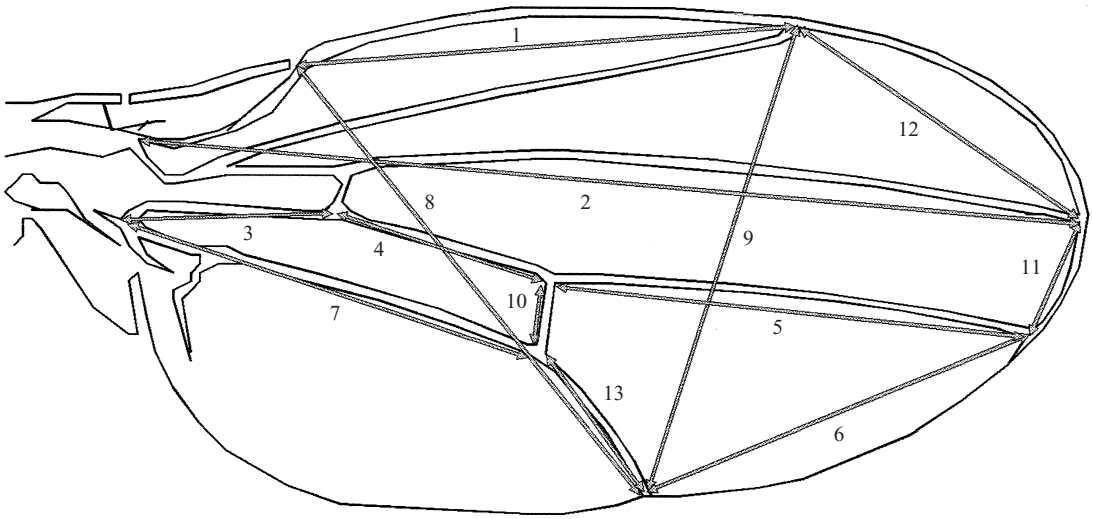

Fig. 1. Position of the 13 different traits measured on the wing.

measurement error. Because of a scaling effect, g and e_i are generally proportional to x, leading to a constant CV when the standard deviation is divided by x. Such is not the case for the measurement error, which introduces a constant, additional variance, independent of x. The weight of e_m is negligible for longer parts of the wing but very important for shorter ones, resulting in a hyperbolic decrease in CVs

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Table 2. *ANOVA on within-line and between-line coefficients of variation of 13 wing traits in 10 isofemale lines of* D. melanogaster *and* D. simulans *grown at seven different temperatures*

		Within-line CV				Between-line CV			
Source	d.f.	MS	*F*	*P*	Variation explained	MS	*F*	*P*	Variation explained
Temperature	6	2·931	13·83	***	4·3	4·515	8·56	***	9·1
Species (2)	1	0·058	0·07	NS	0·0	17·982	5·76	*	6·0
Trait (3)	12	29·587	34·32	***	86·1	9·019	2·80	*	36·4
1 × 2	6	0·502	3·07	**	0·7	6·242	14·42	***	12·6
1 × 3	12	0·211	1·29	NS	3·7	0·527	1·21	NS	12·8
2 × 3	72	0·813	4·98	***	2·4	3·119	7·20	***	12·6
1 × 2 × 3	72	0·163			2·8	0·432			10·5

Temperature and species were considered as fixed, and traits as random. d.f. errors were computed using the Satterthwaite method.
Variation explained is the percentage of total variation explained by the effect.
d.f. degrees of freedom; MS, mean square; *F*, variance ratio.
$*P < 0{\cdot}05$; $**P < 0{\cdot}01$; $***P < 0{\cdot}001$; NS, non-significant.

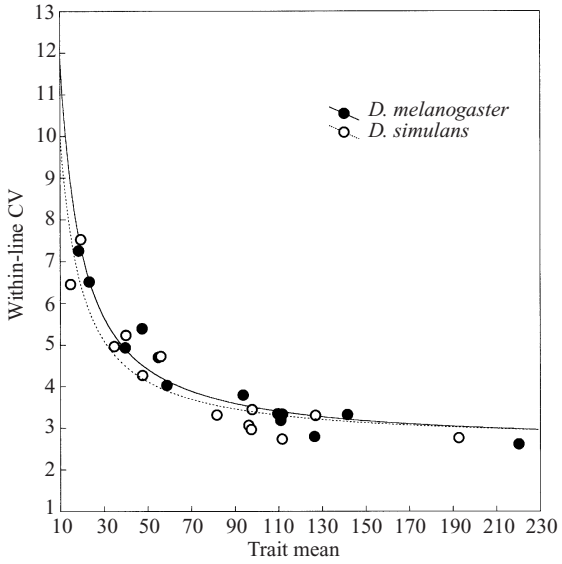

Fig. 2. Relationship between the within-line CV and the mean value of each wing trait in the two species. Data are adjusted to a hyperbolic function for each species. Asymptotic values of CVs are 2·56 and 2·64 for *D. melanogaster* and *D. simulans* respectively.

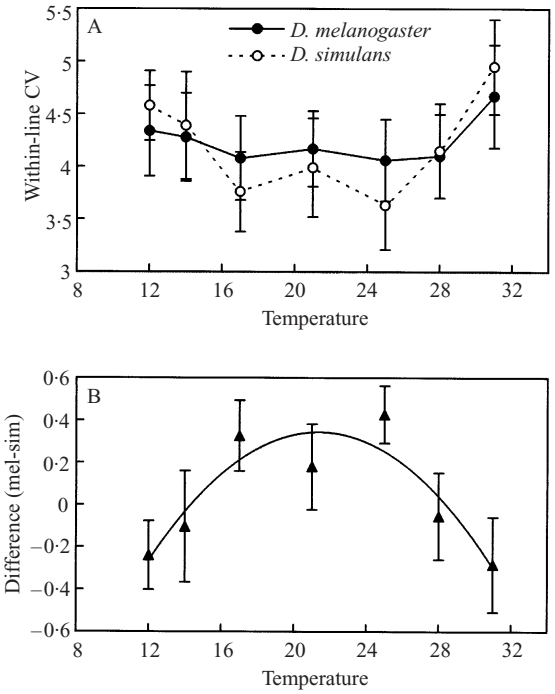

Fig. 3. Relationship between within-line CV and growth temperature in *D. melanogaster* and *D. simulans*. (*A*) Comparison of mean value of 13 traits. (*B*) Analysis of the difference between *D. melanogaster* and *D. simulans*; a quadratic curve is fitted to experimental points. Vertical bars indicate the standard error in both graphs.

according to *x* values (Fig. 2), as previously pointed out by Rohlf *et al.* (1983).

The temperature effect, which explains 4·3% of total variability, is analysed in Fig. 3 *A*. In both species we observed lower values at medium temperatures (17–25 °C) and higher values in more extreme environments (12–14 °C and 28–31 °C). The temperatures of minimum variability, calculated after a quadratic adjustment, are similar in the two species: 20·7 °C for *D. melanogaster* and 21·1 °C for *D. simulans*. The highly significant species–temperature interaction is also shown on the graph: the reactivity to temperature is more pronounced in *D. simulans*, resulting in a stronger curvature of the response curve. We further investigated this phenomenon by calculating for each temperature and trait the difference between CVs of the two species. Differences were negative at extreme temperatures (*D. melanogaster* less variable than *D. simulans*) but positive at middle temperatures (*D. melanogaster* more variable). The resulting concave quadratic curve, shown in Fig. 3 *B*, exhibits a difference

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Table 3. *Genetic variance of 13 wing traits in* D. melanogaster *and* D. simulans *at different growth temperatures*

Trait	12 °C	14 °C	17 °C	21 °C	25 °C	28 °C	31 °C	Mean	SE
D. melanogaster									
1	34·57	36·29	24·07	11·94	11·98	36·09	28·90	26·26	4·04
2	66·54	52·92	29·14	17·10	21·71	59·46	45·50	41·77	7·29
3	5·31	4·52	2·70	1·80	1·36	2·95	2·10	2·96	0·55
4	8·12	8·46	5·35	5·38	3·11	8·07	9·17	6·81	0·84
5	21·67	12·15	6·71	3·66	8·82	14·71	14·54	11·75	2·26
6	18·80	9·07	5·83	5·61	7·94	15·92	16·66	11·40	2·10
7	21·27	18·89	14·84	13·82	9·09	21·63	22·27	17·40	1·87
8	17·83	12·87	6·49	5·37	6·82	14·14	17·01	11·51	1·98
9	22·78	20·09	14·37	6·55	8·68	17·73	13·97	14·88	2·22
10	1·46	0·49	0·99	0·54	0·29	1·47	1·22	0·92	0·18
11	2·45	0·70	0·68	0·33	0·74	0·90	1·03	0·98	0·26
12	9·16	4·48	4·24	4·62	4·07	3·31	4·16	4·86	0·73
13	2·81	1·29	2·30	0·00	0·93	0·91	2·10	1·48	0·37
Mean	17·90	14·02	9·06	5·90	6·58	15·18	13·74		
SE	4·91	4·26	2·50	1·48	1·65	4·66	3·61		
D. simulans									
1	10·46	20·45	17·16	7·07	15·31	12·65	10·70	13·40	1·72
2	21·70	26·69	23·54	26·88	21·26	22·54	14·25	22·41	1·60
3	3·91	3·70	5·39	6·57	6·57	4·42	6·70	5·32	0·50
4	0·94	2·10	0·81	0·53	0·90	1·35	2·07	1·24	0·24
5	2·43	6·31	8·43	4·85	5·35	4·04	0·79	4·60	0·95
6	3·59	3·78	5·82	5·39	4·97	4·23	2·71	4·36	0·42
7	10·00	8·56	8·40	12·83	7·55	9·92	7·89	9·31	0·69
8	9·80	7·98	8·95	6·93	9·13	6·03	4·05	7·55	0·77
9	2·80	3·43	5·37	5·36	6·05	3·16	5·07	4·46	0·49
10	0·53	0·23	0·51	0·54	0·73	0·36	0·50	0·49	0·06
11	0·49	0·51	1·05	1·96	0·69	0·81	0·53	0·86	0·20
12	5·09	5·58	5·16	3·76	0·86	1·85	0·38	3·24	0·83
13	0·78	0·49	0·43	0·43	0·69	0·03	0·08	0·42	0·11
Mean	5·58	6·91	7·00	6·39	6·16	5·49	4·28		
SE	1·68	2·21	1·88	1·96	1·74	1·75	1·24		

maximum at 21·4 °C and the curvature parameter is highly significant. Highly significant effects are due to trait and temperature (two-way ANOVA, not shown).

(ii) *Genetic variability and evolvability*

Genetic variances of wing traits are given in Table 3. As for the within-line variance, major differences were revealed between traits (range 0·42–41·8). With the exception of trait 3, higher values were found in *D. melanogaster* than in *D. simulans*. Genetic coefficients of variation (CVg), which reflect the evolvability of a trait (Houle, 1992), were calculated and submitted to ANOVA (Table 2). All main effects and interactions were found to be significant, with the exception of the temperature–trait interaction. The main part of the total variation (36·4 %) was due to traits. Direct temperature effects explained 9·1 % of the total genetic variance (higher variability at extreme temperatures) and the species–temperature interaction accounted for almost 12·6 %.

Traits effects were analysed by considering the relationship between CVg and mean value of each trait (Fig. 4). Negative correlations were found for each species, i.e. $r = -0{\cdot}52$ for *D. melanogaster* and $r = -0{\cdot}51$ for *D. simulans*. This phenomenon is akin

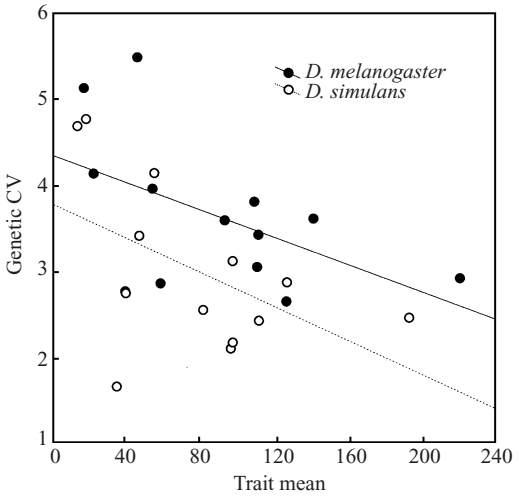

Fig. 4. Relationship between genetic CVs and mean value of 13 traits in two species. Linear regression lines are shown. $r = -0{\cdot}52$ ($P = 0{\cdot}067$) in *D. melanogaster* and $r = -0{\cdot}51$ ($P = 0{\cdot}073$) in *D. simulans*.

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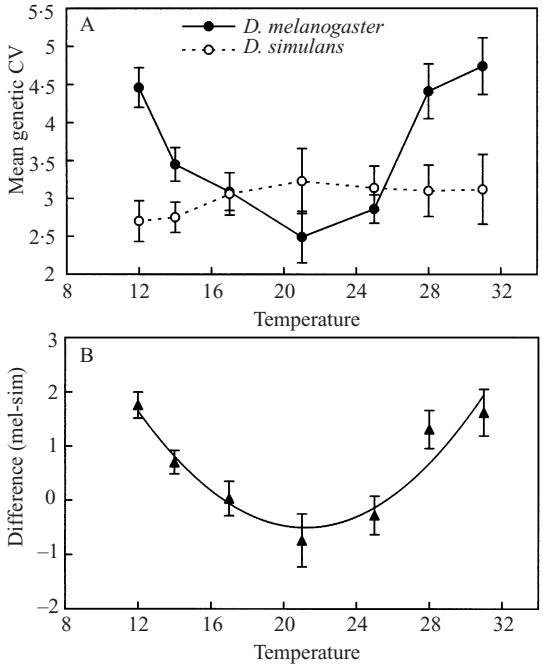

Fig. 5. Relationship between genetic CV and growth temperature. (*A*) Comparison of mean values of 13 traits. (*B*) Analysis of the difference between *D. melanogaster* and *D. simulans*. A quadratic curve is fitted to experimental points. Vertical bars indicate the standard error in both graphs.

to what was observed for the within-line variance, but is difficult to interpret in the same way. There is no reason to believe that the measurement errors, which are relatively more important on smaller traits, should also differentiate the lines. Moreover, at least in *D. simulans*, there are two short traits which have a high CVg, above 4·5%, but another one which has a very low CVg of 1·6%. It is therefore possible that the variations shown in Fig. 4 reflect a real biological phenomenon, that is an overall tendency for longer traits to be genetically less variable than shorter ones.

Species and temperature effects on CVg are shown graphically in Fig. 5*A*, illustrating the higher genetic variability in *D. melanogaster* and the difference in the shapes of the response curves. Data for each species were submitted separately to ANOVA (not shown) and, in each case, the temperature effect remained significant. In other words, genetic variability was significantly minimum in *D. melanogaster* at intermediate temperatures (20·7 °C) but maximum in *D. simulans* (24·7 °C). We also calculated and analysed the square of the CVg, as another possible estimate of evolvability (Houle, 1992). Results, not shown, provided basically the same information as the CVg. The interspecific difference was further investigated by considering, for each CVg, the deviation between the two species (*D. melanogaster* minus *D. simulans*). We obtained a highly significant convex quadratic curve (Fig. 5*B*), indicating that *D. melanogaster* is genetically more variable at extreme temperatures than its sibling. This result contrasts with the within-line CV, which exhibits a concave quadratic difference (Fig. 3*B*).

(iii) *Isofemale heritability*

Isofemale heritabilities, defined as the intraclass correlations, were calculated for each trait and temperature and are given in Table 4. ANOVA on this data set (Table 5) indicated that all main effects and interactions were significant except the temperature–trait interaction. Traits showed the largest effect (30·2%) while the direct effect of temperature was quite small and accounted for only 4·2% of the total variation. A major temperature–species interaction accounted for 23·6% of the total variation.

The significant interspecific difference corresponds to a higher heritability in *D. melanogaster* (on average $0{\cdot}43 \pm 0{\cdot}03$) than in *D. simulans* ($0{\cdot}36 \pm 0{\cdot}03$). Significant variations were observed between traits (range 0·26–0·54 in *D. melanogaster* and 0·12–0·45 in *D. simulans*). The trait effect was further analysed by correlating heritabilities and mean values (Fig. 6). In both cases, significant positive correlations were obtained, that is $r = 0{\cdot}75$ ($P < 0{\cdot}01$) in *D. melanogaster* and $r = 0{\cdot}61$ ($P < 0{\cdot}05$) in *D. simulans*. Smaller wing dimensions exhibit lower heritabilities and vice versa. This phenomenon is presumably due to a predominant effect of measurement errors, which artificially increase the denominator of the intraclass correlation in shorter traits. Indeed, as seen in Fig. 4, there is some indication that short traits might be genetically more variable than longer ones.

For investigating the temperature effect and the temperature–species interaction, isofemale heritabilities were averaged over traits and plotted against growth temperatures (Fig. 7*A*). In *D. melanogaster*, heritability values increase at both high and low extreme temperatures, and the resulting curve is convex with a minimum at 20·8 °C. By contrast, in *D. simulans*, an opposite trend is observed: the average reaction norm is concave with a maximum at 21·8 °C. The opposite shapes of the response curves account for the high species–temperature interaction. When each species was separately subjected to ANOVA, the temperature effect was still highly significant ($P < 10^{-5}$, not shown). As in previous sections, we considered the mean difference between species (Fig. 7*B*) and found a concave quadratic curve, which visualizes the higher heritability of *D. melanogaster* at extreme temperatures and the higher heritability of *D. simulans* at medium temperatures.

(iv) *Heritability and evolvability*

Evolvability (CVg, Fig. 5*A*) and heritability (Fig. 7*A*) show similar response curves according to growth

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Table 4. *Intraclass correlation coefficients of 13 wing traits in* D. melanogaster *and* D. simulans *at different growth temperatures*

Trait	12 °C	14 °C	17 °C	21 °C	25 °C	28 °C	31 °C	Mean	SE
D. melanogaster									
1	0·57	0·59	0·46	0·37	0·38	0·70	0·60	0·52	0·05
2	0·62	0·61	0·45	0·29	0·45	0·72	0·60	0·54	0·05
3	0·50	0·49	0·34	0·27	0·21	0·31	0·25	0·34	0·04
4	0·48	0·56	0·39	0·53	0·34	0·61	0·59	0·50	0·04
5	0·62	0·39	0·37	0·19	0·40	0·64	0·64	0·46	0·07
6	0·59	0·37	0·29	0·26	0·41	0·63	0·64	0·46	0·06
7	0·59	0·61	0·51	0·47	0·43	0·64	0·64	0·56	0·03
8	0·60	0·42	0·33	0·30	0·38	0·60	0·59	0·46	0·05
9	0·55	0·55	0·42	0·31	0·44	0·69	0·56	0·50	0·05
10	0·49	0·19	0·32	0·26	0·14	0·51	0·39	0·33	0·06
11	0·34	0·21	0·21	0·12	0·33	0·39	0·39	0·29	0·04
12	0·56	0·32	0·37	0·33	0·46	0·40	0·45	0·42	0·03
13	0·38	0·17	0·40	−0·01	0·22	0·24	0·43	0·26	0·06
Mean	0·53	0·42	0·38	0·28	0·35	0·55	0·52	0·43	
SE	0·02	0·05	0·02	0·04	0·03	0·04	0·04	0·03	
D. simulans									
1	0·25	0·50	0·54	0·34	0·57	0·49	0·35	0·44	0·05
2	0·31	0·50	0·47	0·53	0·58	0·46	0·30	0·45	0·04
3	0·30	0·23	0·53	0·54	0·54	0·46	0·49	0·44	0·05
4	0·16	0·30	0·19	0·09	0·17	0·32	0·30	0·22	0·03
5	0·11	0·39	0·48	0·44	0·50	0·41	0·09	0·34	0·06
6	0·23	0·33	0·41	0·49	0·54	0·40	0·27	0·38	0·04
7	0·40	0·42	0·48	0·53	0·49	0·49	0·37	0·46	0·02
8	0·41	0·39	0·51	0·48	0·60	0·50	0·27	0·45	0·04
9	0·15	0·27	0·41	0·42	0·56	0·32	0·39	0·36	0·05
10	0·40	0·14	0·38	0·36	0·44	0·32	0·40	0·35	0·04
11	0·14	0·13	0·33	0·47	0·35	0·32	0·26	0·29	0·04
12	0·45	0·51	0·57	0·44	0·25	0·31	0·13	0·38	0·06
13	0·15	0·08	0·12	0·16	0·27	0·02	0·02	0·12	0·03
Mean	0·27	0·32	0·42	0·41	0·45	0·37	0·28	0·36	
SE	0·03	0·04	0·04	0·04	0·04	0·04	0·04	0·03	

Table 5. *Result of a three-way mixed model ANOVA on intraclass correlation coefficients of 13 wing traits in 10 isofemale lines of* D. melanogaster *and* D. simulans *grown at seven different temperatures*

Source	d.f.	MS	*F*	*P*	Variation explained
Temperature (1)	6	0·030	4·04	***	4·2
Species (2)	1	0·245	7·81	*	5·7
Trait (3)	12	0·108	3·64	*	30·2
1 × 2	6	0·169	18·74	***	23·6
1 × 3	72	0·007	0·83	NS	12·5
2 × 3	12	0·031	3·46	***	8·7
1 × 2 × 3	72	0·009			15·1

Temperature and species are fixed, and traits are random. d.f. errors are computed using the Satterthwaite method.
Variation explained is the percentage of total variation explained by the effect.
d.f., degrees of freedom; MS, mean square; *F*, variance ratio.
*$P < 0·05$; **$P < 0·01$; ***$P < 0·001$.

temperature, and both contrast the two species, with the minimum genetic variability in the middle of the thermal range in *D. melanogaster* but the maximum in *D. simulans*. Moreover, both CVg and heritability harbour the genetic variance at the numerator, so that a positive correlation might be expected. We analysed

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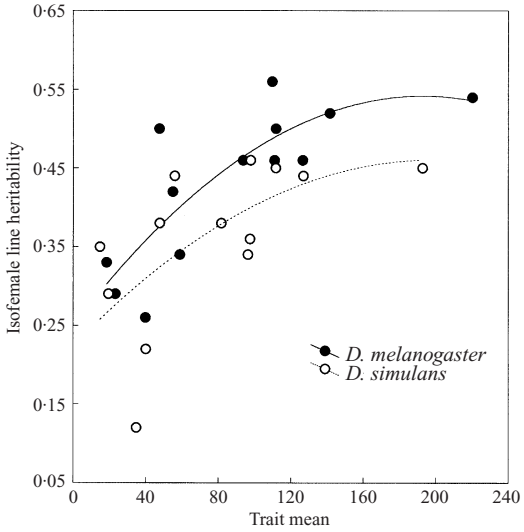

Fig. 6. Increase of isofemale heritability (intraclass correlation) according to mean value of 13 linear traits. Values obtained for each trait at different temperatures were averaged.

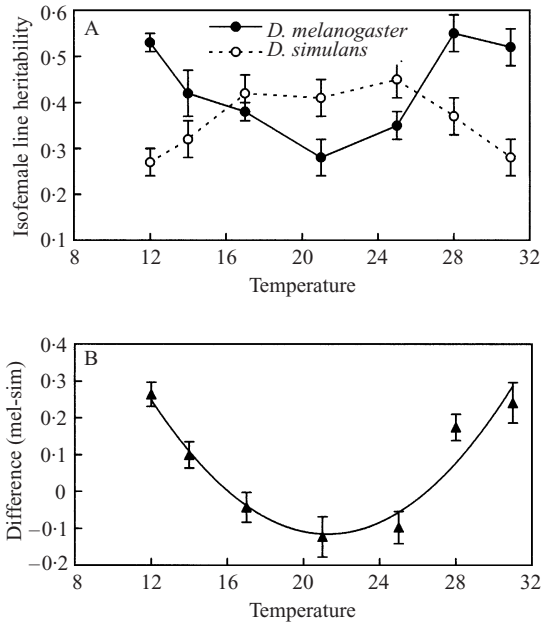

Fig. 7. Relationship between isofemale heritability and growth temperature. (*A*) Variation in mean values of 13 wing traits in the two species. (*B*) Analysis of the difference between *D. melanogaster* and *D. simulans*; a quadratic curve is fitted to the experimental points. Vertical bars are the standard error in both graphs.

the covariation of heritability and evolvability (Fig. 8) and found a complete lack of correlation: $r = -0{\cdot}01$ in *D. melanogaster* and $r = 0{\cdot}18$ in *D. simulans*. This confirms the argument of Houle (1992) that these two characteristics provide different biological information and should both be considered in evolutionary studies.

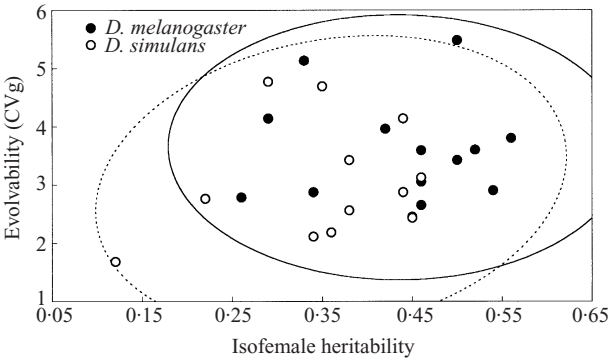

Fig. 8. Scatterplot of isofemale heritability against evolvability for 13 wing traits in the two species. Ellipses of 90% confidence area are shown to help visualize the data.

4. Discussion and conclusions

In *Drosophila*, wing size and wing shape are receiving increasing attention from quantitative, developmental and evolutionary geneticists. The overall stability of the structure permits an unambiguous identification of homologous points. A diversity of methods has been used to investigate wing variation, including the analysis of the overall contour (Cavicchi *et al*., 1991), the measurement of angular values (Bitner-Mathé & Klaczko, 1999), the relative displacement of homologous points (Gilchrist *et al*., 2000) and the proportion of some linear measurements (Loeschcke *et al*., 1999; Birdsall *et al*., 2000; Huey *et al*., 2000). We investigated the phenotypic and genetic variability of 13 linear dimensions in 10 isofemale lines of two sympatric sibling species grown at seven different constant temperatures.

Mean values of these traits were previously shown (Moreteau *et al*., 1998) to react quite differently to temperature and to exhibit reaction norms with different shapes. In other words the traits are partly independent and do not provide redundant genetic information. The same conclusion may be drawn from the analysis of genetic correlations (unpublished data). In this paper, we focused attention on the genetic architecture of the natural populations of these species, and its variation according to the thermal environment. For such a comparison, two strategies are possible: either investigating a very large number of isofemale lines or, as in the present work, a series of different, but similar traits, which can to some extent be pooled and averaged.

Our major conclusion has been that, when investigated over their complete thermal range, both species exhibited significant variation in heritabilities, but in opposite directions: in *D. melanogaster* an increased heritability was observed at extreme (low or high) temperatures, while in *D. simulans*, heritability was maximum in the middle of the thermal range.

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Moreover, since phenotypic variability could be divided into a within-line and a between-line component, it was possible to analyse separately the effects of growth temperature upon each of them.

The within-line variance mainly estimates a non-heritable, environmental component of the variability. Since the mean values of the traits were very different, the variances were also highly heterogeneous and it was necessary to standardize the data to the mean by considering the coefficient of variation. However, the CVs remained strongly dependent on the mean, according to a hyperbolic function. Such a phenomenon has already been observed in several investigations and has sometimes been called the Kluge–Kerfoot phenomenon (Kluge & Kerfoot, 1973). In agreement with later studies (Rohlf *et al*., 1983; Houle, 1992) we interpret this observation as a consequence of measurement errors. Such errors are generally neglected in quantitative genetics investigations, but they can be important when comparing small and large dimensions. A re-analysis of data obtained by Noach *et al*. (1996) for various dimensions of *D. melanogaster* also suggested such a hyperbolic relationship. In spite of this experimental problem we were able to analyse the effects of growth temperature upon the environmental variability. In the two species, similar response curves have been obtained, that is an increase in the variability in extreme, stressful conditions (low or high temperature), in agreement with apparently most previous investigations dealing either with total wing and thorax length in the same species (David *et al*., 1994; Morin *et al*., 1996) or with other traits in other species (Barker & Krebs, 1995; Imasheva *et al*., 1997, 1998). Such a general phenomenon is generally considered as an impairment of developmental canalization (Waddington, 1957) or of phenotypic homeostasis (Lerner, 1954) under unfavourable conditions, that is an increase in the developmental noise. A comparison of the two species showed, however, that *D. simulans* was less variable than its sibling at medium temperatures but more variable at extreme temperatures. In other words, *D. simulans* seems more reactive to its thermal environment.

Genetic variance among lines was also highly variable according to trait mean value and standardized as a genetic coefficient of variation. CVg is an interesting measure since it provides some information about the possible response of a trait to directional selection, that is on its evolvability (Houle, 1992). In agreement with Houle (1992) we found that evolvability and heritability were not correlated and thus provided independent information. For wing dimensions, evolvability appeared to be significantly lower in *D. simulans* than in *D. melanogaster*, since mean CVg values were respectively $3{\cdot}12 \pm 0{\cdot}45$ and $4{\cdot}74 \pm 0{\cdot}37$. This may be correlated with the fact that *D. simulans* is known to be far less differentiated into geographic morphological races than its sibling (Capy *et al*., 1993). There was a slight but significant tendency for CVg to be larger in shorter traits. This relationship does not seem to be linked to measurement errors and might reflect a real biological phenomenon, which however deserves further investigations. Interestingly, heritability (intraclass correlation) was positively correlated with mean trait value, again confirming its independence from evolvability.

The increase in evolvability and heritability under stressing conditions which has been observed in *D. melanogaster*, agrees with most previous observations, as indicated in Section 1, and confirms some theoretical predictions. Data on *D. simulans* lead, however, to an opposite conclusion, showing at least the danger of theoretical generalization with an insufficient set of empirical data. In the case of *D. melanogaster*, we consider that our observations confirm previous investigations (see Section 1) and correspond to a general property of that species. In further support of this proposal, we found recently a remarkable stability of the reaction norms of size characters in two samples of 10 lines collected in the same place over a 5 year interval (Karan *et al*., 1999*b*).

D. simulans remains, however, a less studied species and more extensive investigations are needed. It remains possible that the increase in genetic variability at medium temperatures which has been observed here on a sample of only 10 isofemale lines, reflects some sampling effect of these lines and would not be confirmed by more extensive studies. We may indicate, however, that on a different sample of the same species (Morin *et al*., 1996), higher heritabilities were already observed at medium temperatures for total wing length and thorax length. Results were especially clear for the thorax, with an average heritability of $0{\cdot}392 \pm 0{\cdot}030$ between 17 and 25 °C, and a much lower value ($0{\cdot}190 \pm 0{\cdot}038$) at extreme temperatures (12–14 °C and 28–31 °C).

For understanding and modelling the evolution of phenotypic plasticity (Via *et al*., 1995) it would be most important to be sure that closely related species may exhibit different reaction norms for the genetic variance, heritability and evolvability of quantitative traits.

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