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Phenotypic plasticity of body pigmentation in *Drosophila*: correlated variations between segments

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Abstract – Phenotypic plasticity of body pigmentation (the last three abdominal segments and the mesothorax) was investigated as a function of growth temperature in *Drosophila melanogaster* and *Drosophila simulans*. Two populations of each species were analysed, from two French localities with different climatic conditions. For each population, ten isofemale lines were reared at temperatures ranging from 14 to 31 °C. Two methods were used and compared to estimate genetic correlations (r_g) between segments, a simple method using directly the family mean values (r_m) and a theoretically better method correcting variances and covariances for family size (r_c). Both methods produced very similar data but the first one (r_m) was preferred because it allowed an estimate of r_g in all cases. Genetic and phenotypic correlations decreased regularly with distance between body segments, revealing an antero-posterior gradient: the extension of dark pigmentation is determined by increasingly different genetic systems in more distant segments. Genetic correlations were substantially larger than phenotypic correlations, in opposition to Cheverud's conjecture, although the two sets of values were highly correlated. © Inra/Elsevier, Paris

D. melanogaster / D. simulans / genetic correlation / Cheverud's conjecture / isofemale lines

Résumé – **Plasticité phénotypique de la pigmentation corporelle chez *Drosophila* : variations corrélées entre segments.** La plasticité phénotypique de la pigmentation corporelle (trois derniers segments abdominaux et mésothorax) a été étudiée en fonction de la température chez *Drosophila melanogaster* et *Drosophila simulans*. Pour chaque espèce, deux populations ont été analysées, provenant de deux localités françaises ayant

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des conditions climatiques différentes. Pour chaque population, dix lignées isofemelles ont été élevées à des températures comprises entre 14 et 31 °C. Deux méthodes ont été utilisées et comparées pour estimer les corrélations génétiques (r_g) entre segments, une méthode simple utilisant directement les valeurs moyennes des familles (r_m) et une méthode théoriquement meilleure, corrigeant les variances et les covariances par la taille de la famille (r_c). Les deux méthodes ont produit des données similaires mais la première (r_m) a été préférée, car elle permet une estimation dans tous les cas. Les corrélations génétiques et phénotypiques diminuent quand on compare des segments plus distants, révélant un gradient antéro-postérieur. L'extension de la pigmentation noire est déterminée par des systèmes génétiques de plus en plus différents dans des segments plus éloignés. Les corrélations génétiques sont significativement supérieures aux corrélations phénotypiques, ce qui est en opposition avec la conjecture de Cheverud, bien que les deux valeurs soient hautement corrélées. © Inra/Elsevier, Paris

D. melanogaster / D. simulans / corrélation génétique / conjecture de Cheverud / lignées isofemelles

1. INTRODUCTION

Phenotypic plasticity, the capacity of a single genotype to produce different phenotypes in different environments, is a general property of living organisms. In many cases, for example enzymatic adaptation in bacteria or the production of wingless or winged forms in aphids, the adaptive significance of the polymorphism is obvious [27, 32, 36, 39]. The interpretation is, however, far more difficult when a continuous environmental gradient results in continuous variation of the phenotype, the response curve being called the norm of reaction. Numerous cases have been investigated in plants and animals, unravelling two major, but unsolved questions. Are there specific genes acting on the shape of the norms, independently of the mean trait value? Is the shape of the norms acted upon by natural selection, thus exhibiting a specific adaptive value?

Body pigmentation in numerous ectotherm species is known to exhibit broad variation, resulting either from genetic polymorphism or phenotypic plasticity. In the latter case, darker individuals are generally observed at low temperatures [1, 8, 9, 15, 16, 18, 19, 22, 25, 40]. Increased melanization at lower temperatures is generally thought to be adaptive for thermoregulation: being darker will favor the absorption of light radiation and thus improve metabolic activity at low temperature; the reverse being true at high temperature.

In *Drosophila melanogaster* and related species, phenotypic plasticity of pigmentation can be quantified on the mesothorax (a dark pattern with a trident shape) [2, 8] and on the tergites of female abdomen segments [9, 13]. The occurrence of latitudinal clines for the thoracic trident is a major argument in favor of the adaptive significance of pigmentation variations [2, 8, 24]. For abdomen pigmentation, it was also shown that the shapes of reaction norms were different in two French localities differing by their climatic conditions [13]. Reactivity to low developmental temperature was stronger in populations living in the place with more marked seasonal variation and colder winters. The fact that similar results were observed in two sibling species (*D. melanogaster* and *D. simulans*) enhanced the likelihood that this variation in reaction norm shape was adaptive. In these two species, the shapes of reaction norms are also different according to body segment [9, 13]. This

suggests an interaction between developmental genes which specify the formation of adult segments and genes which react to temperature and determine the extension of the black pigment.

The procedure of isofemale lines is not the best method for investigating the genetic architecture of quantitative traits. This procedure has proved however to be extremely useful in ecological genetics for the description of quantitative characters of natural populations [14, 23, 26]. Once isofemale lines have been analysed, we need to extract the maximum information from experimental observations. With respect to heritability, the coefficient of intraclass correlation is generally used as an approximation providing an 'isofemale heritability' [3, 17]. Concerning genetic correlation, the situation is more ambiguous. In various papers [17] genetic correlations have been estimated using a software package, but the exact procedure was not described. On the other hand, the problem was clearly analysed by Via [37], but several solutions were considered.

In the present paper, we accept the inconveniences of isofemale lines, adopt a pragmatic approach, and address three main issues. a) What is the best way for estimating genetic correlations from isofemale lines data? b) To what extent are different genes involved in determining the extension of black pigment in different body segments? c) We tested Cheverud's conjecture [5, 20, 29] that phenotypic correlations are similar to genetic correlations. In other words, phenotypic correlations, which are easier to measure, could be convenient predictors of genetic correlations.

2. MATERIALS AND METHODS

2.1. Populations and experimental procedures

Sympatric French populations of *D. melanogaster* and *D. simulans* were collected in a city garden in Villeurbanne near Lyon and a vineyard in Grande Ferrade near Bordeaux. Wild living females were isolated in culture vials to establish isofemale lines. After offspring emergence, ten lines of each species and locality were randomly taken, and from each line ten adult pairs were used as parents of the studied flies. This procedure is necessary to obtain a sufficient progeny number [23]. Investigated flies were thus the second laboratory generation. These parental flies were allowed to oviposit for a few hours in successive vials containing a killed yeast medium [6]. Vials with eggs were then transferred at one of six experimental temperatures (14, 17, 21, 25, 28 and 31 °C) chosen to cover almost the full thermal range compatible with sufficient viability [7]. With this procedure, larval density was kept around 150 per vial, and the use of a high nutrient food reduced crowding effects. On emergence, adults were transferred to fresh food and examined a few days later. From each line at each temperature, ten females were randomly taken and measured.

2.2. Pigmentation scores

We analysed pigmentation on the mesonotum and on the last three abdominal segments. Thoracic pigmentation (a dark area with a trident shape, designated as trid) can be analysed in males and females [8] while abdomen pigmentation is variable in females only. In the present work, only females were analysed. The

extension of black pigment on the last three abdominal tergites (segments 5, 6 and 7) was estimated visually; 11 phenotypic classes were used (see David et al. [9] for details), ranging from 0 (completely yellow) to 10 (completely dark). For the thoracic trident, only four phenotypic classes were used, ranging from 0 (no visible trident) to 3 (dark trident) (see David et al. [8] for details). For comparing thorax and abdominal pigmentation, we standardized their possible range of variation. The trident pigmentation score was thus multiplied by 3.33, so that variability ranged between 0 and 10.

Pigmentation variation on either thorax or abdomen is continuous and the establishment of phenotypic classes introduces the possibility of some bias according to observer. We have always been careful about this problem, and verified that identical average scores would be obtained either when the same observer is looking twice at the same flies or when two observers compare their data ([8] and unpublished observations). The same conclusion arises from the high genetic repeatability which is found when successive generations of the same isofemale lines are investigated [14].

2.3. Data analysis

Analyses were performed with Statistica [33] and SAS® [31] software.

With available data (four segments) we calculated six possible pairwise correlations among segments. For each pair of characters, each temperature and each segment, we calculated three different correlations: the total, phenotypic correlation (r_p; 100 flies); the correlation between family means (r_m; 10 families) and the within family correlation (r_w; 10 flies per family).

It was suggested [37] that in many cases r_m could be used as an approximation of the genetic correlation r_g. By simulation, Roff and Preziosi [30] showed however that equating r_m to r_g would be biased unless family size n would be sufficiently large (> 20). Since in our case family size was less ($n = 10$), we calculated a corrected value (r_c) between family means, for a better estimate of the genetic correlation, according to the expression:

$$r_c = \frac{\mathrm{cov}_{m(XY)} - 1/n\,\mathrm{cov}_{w(XY)}}{\sqrt{(\mathrm{var}_{m(X)} - 1/n\,\mathrm{var}_{w(X)})(\mathrm{var}_{m(Y)} - 1/n\,\mathrm{var}_{w(Y)})}}$$

where X, Y designate the covariates (two segments), cov_m and cov_w are the covariances between family means and within families, and var_m and var_w the corresponding variances of each variable.

In the present work, numerous correlation values were available for example between the ten females of each line grown at each temperature. These values were then submitted to ANOVA, after z transformation. Results helped us to decide how to pool the data for a more synthetic presentation. For example, in absence of a significant temperature effect, we calculated a single value for each isofemale line, as carried out in *table III*. Values of the ten lines could then be averaged and a standard error provided. Comparisons of distributions were made with a non-parametric Mann-Whitney test. Since the objective of this paper was focused on biological conclusions, detailed statistical comparisons are not presented.

3. RESULTS

3.1. An antero-posterior gradient

Darker flies are obtained at lower growth temperatures but the various segments do not react in the same way and exhibit different levels of plasticity. This interaction between temperature and body segments is illustrated *figure 1* for the two species. In *D. simulans*, a regular antero-posterior gradient of increasing darkness is observed at low temperatures (14–21 °C). At higher temperatures an irregularity is observed since segment 6 is darker than segment 7. In *D. melanogaster*, the antero-posterior gradient is not found for two reasons. One is that the thoracic trident is darker than in *D. simulans*. The second is that segment 6 is darker than segment 7 at all temperatures.

Phenotypic correlations between any pair of segments were calculated for each temperature and population (100 flies in each sample, *table I*) and submitted to an ANOVA (not shown) after a z transformation. Higher correlations were observed between adjacent segments while lower values were found when more distant body segments are considered ($F_{(5, 67)} = 66.49$, $P < 0.001$). Average r_p values decrease from about 0.50 when adjacent segments were correlated (5–6 and 6–7) down to a non-significant 0.07 when correlating segment 7 with trident (*figure 2*). There are higher correlations in *D. melanogaster* than in its sibling ($F_{(1,67)} = 42.24$, $P < 0.001$), higher values in Villeurbanne than in Bordeaux especially in *D. melanogaster* ($F_{(1.67)} = 9.96$, $P < 0.01$), and higher correlations at low temperatures ($F_{(5.67)} = 5.97$, $P < 0.001$). Two other correlations were calculated and analysed: the within-line correlation (r_w) (*table III*) and the correlation between mean values of the isofemale lines (r_m) (*table II*). All values are significantly superior to zero with the exception of thorax-segment 7 (interval 6, all correlations), and the thorax-segment 6 (interval 5) for the within-line correlations. In each case significant variations were also observed (*figure 2*) corresponding to decreasing correlations with increasing segmental distance, thus confirming the antero-posterior gradient.

3.2. Genetic correlations

As stated in the method section, the correlation between family means (r_m) should be corrected when the family size is less than 20. We calculated these corrected values r_c, and compared them to r_m. Average values were found to be very similar and not statistically different (*D. melanogaster*: $r_c = 0.547 \pm 0.0383$, $r_m = 0.539 \pm 0.0396$, $n = 64$; *D. simulans*: $r_c = 0.441 \pm 0.071$, $r_m = 0.408 \pm 0.0634$, $n = 40$). Moreover, variations of r_c and r_m were themselves highly correlated (*figure 3*). Finally, numerous r_c could not be estimated because of either null or negative variances, or of values greater than 1. Over the whole data set 126 r_m were available but only 104 r_c. We thus decided to use r_m as a better general estimate of r_g (*table II*) and submitted them to an ANOVA (not shown) after a z transformation.

These values decreased along the antero-posterior gradient (*figure 2*) ($F_{(5,67)} = 55.82$, $P < 0.001$). Genetic correlations were on average higher in *D. melanogaster*

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D simulans
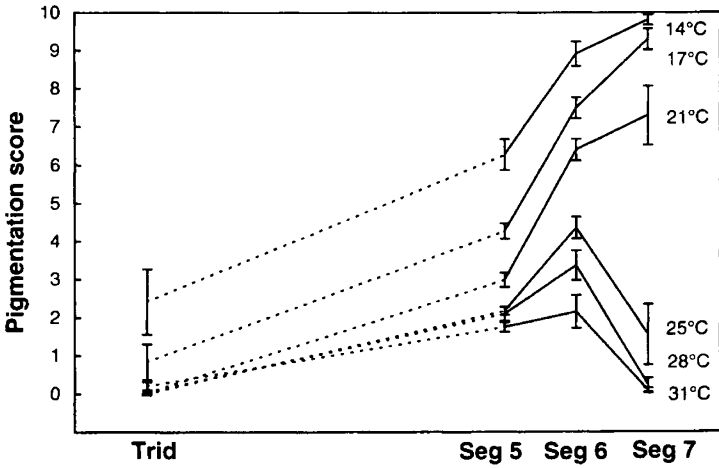
D melanogaster
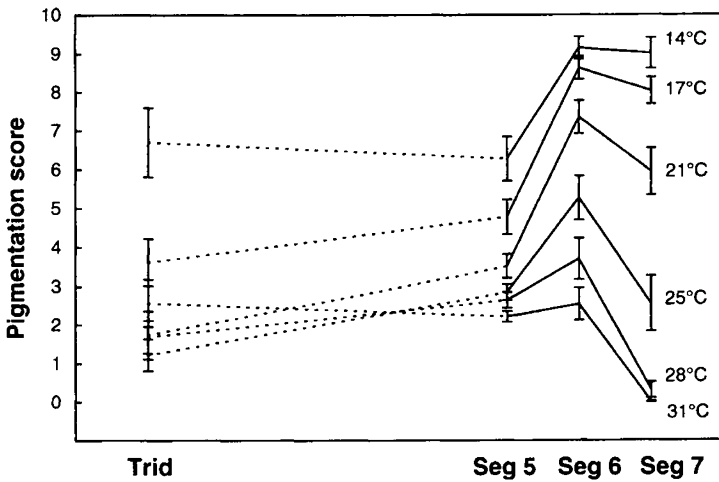
Figure 1. Variation of pigmentation score in different body segments (head is on the left) according to temperature in each species. Original values of trident pigmentation were multiplied by 3.33 in order to have the same variation range as for abdominal segments.

Table I. Coefficients of phenotypic correlation calculated at each temperature ($n = 100$). Threshold values: 0.20 for $P = 0.05$, 0.26 for $P = 0.01$ and 0.32 for $P = 0.001$.

	5–6	5–7	6–7	5–trid	6–trid	7–trid
		D. melanogaster Bordeaux				
14 °C	0.59	0.40	0.71	0.22	0.09	−0.11
17 °C	0.65	0.54	0.84	0.16	0.02	0.07
21 °C	0.64	0.43	0.70	0.05	−0.08	0.003
25 °C	0.57	0.38	0.59	0.14	0.09	0.03
28 °C	0.55	0.23	0.45	0.08	0.04	−0.03
31 °C	0.46	−0.02	0.21	0.10	0.01	0.02
Mean ± s.e.	0.58 ± 0.03	0.33 ± 0.08	0.58 ± 0.09	0.13 ± 0.03	0.03 ± 0.03	−0.003 ± 0.03
		D. melanogaster Villeurbanne				
14 °C	0.67	0.55	0.77	0.59	0.59	0.51
17 °C	0.59	0.54	0.70	0.42	0.36	0.23
21 °C	0.68	0.55	0.74	0.31	0.27	0.17
25 °C	0.66	0.36	0.67	0.25	0.10	0.08
28 °C	0.60	0.19	0.30	0.32	0.17	0.16
31 °C	0.51	n.c.	n.c.	0.43	0.30	n.c.
Mean ± s.e.	0.62 ± 0.03	0.44 ± 0.07	0.64 ± 0.09	0.39 ± 0.05	0.30 ± 0.07	0.23 ± 0.07
		D. simulans Bordeaux				
14 °C	0.57	0.35	0.63	0.21	−0.09	−0.14
17 °C	0.45	0.41	0.56	0.04	0.002	−0.25
21 °C	0.47	0.23	0.59	0.03	0.01	−0.01
25 °C	0.25	0.17	0.53	n.c.	n.c.	n.c.
28 °C	0.65	0.24	0.31	0.15	0.20	0.77
31 °C	0.65	0.16	0.13	0.06	0.09	−0.05
Mean ± s.e.	0.51 ± 0.06	0.26 ± 0.04	0.46 ± 0.08	0.10 ± 0.04	0.04 ± 0.05	0.06 ± 0.18
		D. simulans Villeurbanne				
14 °C	0.50	n.c.	n.c.	0.23	0.10	n.c.
17 °C	0.52	0.33	0.51	−0.06	−0.06	−0.06
21 °C	0.39	0.22	0.53	n.c.	n.c.	n.c.
25 °C	0.39	0.21	0.63	n.c.	n.c.	n.c.
28 °C	0.58	0.12	0.27	n.c.	n.c.	n.c.
31 °C	0.34	0.03	0.30	0.10	0.15	0.04
Mean ± s.e.	0.45 ± 0.04	0.18 ± 0.05	0.45 ± 0.07	0.09 ± 0.08	0.06 ± 0.06	−0.01 ± 0.05

n.c.: not calculated because of a null variance in one trait. The six possible pairwise correlations between segments are indicated as follow: 5, 6 and 7: abdomen segments 5, 6 and 7; trid: thoracic trident (mesonotum).

than in *D. simulans* (0.54 versus 0.33) ($F_{(1,67)} = 37.35$, $P < 0.001$) and higher in Villeurbanne than in Bordeaux in *D. melanogaster* (0.65 versus 0.44) ($F_{(1,67)} = 8.18$, $P < 0.001$).

3.3. Phenotypic correlations and Cheverud's conjecture

The phenotypic correlation integrates a genetic and an environmental component of covariation. Environmental components were estimated as the within-line

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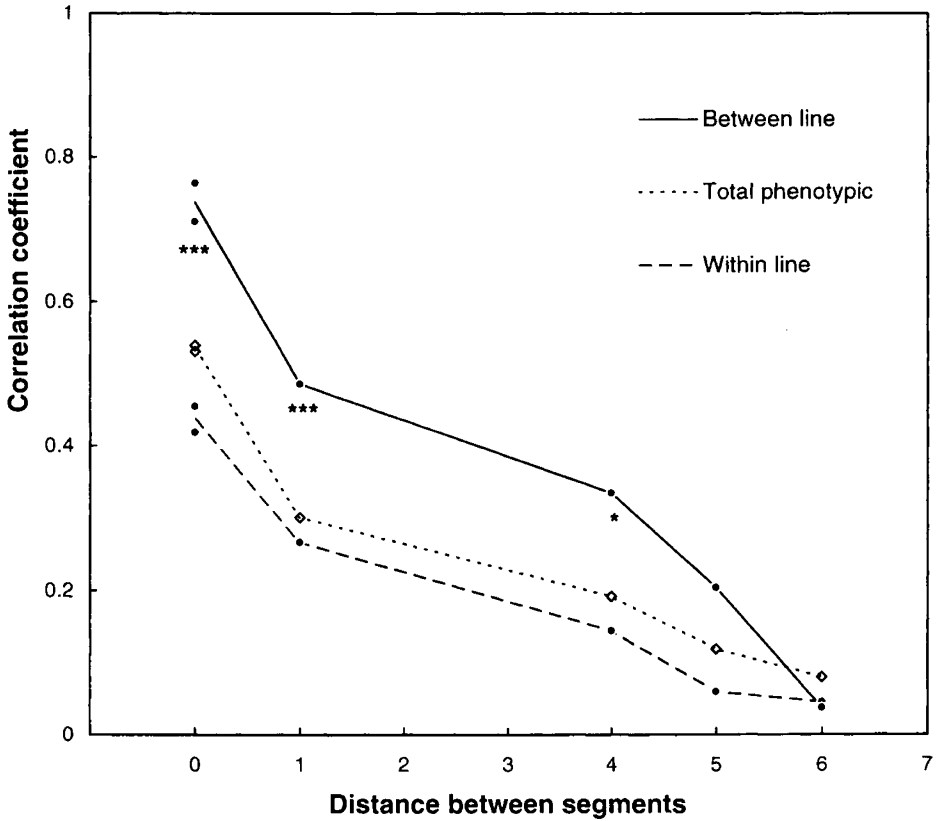
Figure 2. Relationship between average correlation and the distance between correlated segments. Adjacent segments (5–6 or 6–7) were given a distance of zero. Three different correlations were calculated: total phenotypic, within-lines and between-lines which estimates the genetic correlation (see text for details). The within- and between-lines values were compared with a non-parametric Mann-Whitney test: significant differences are indicated on the graph: ***: $P < 0.001$, **: $P < 0.01$.

correlation. The correlations between segments for the ten females reared in the same vial were calculated for each population, line and temperature. In many cases these correlations could not be calculated because either one average value (mainly the trident of *D. simulans*) or a variance (at extreme temperatures) was null. For each line, correlations were averaged over temperatures, and mean values are given in *table III*. Average within-line correlations were highly variable between segments, illustrating again the antero-posterior gradient (*table III, figure 2*).

Cheverud's conjecture [5] states that phenotypic correlations might be convenient approximations of genetic correlations. This requires 1) that the two sets of correlations be highly correlated and 2) that the difference between them be small. Our data clearly show that the two types of correlations were highly correlated
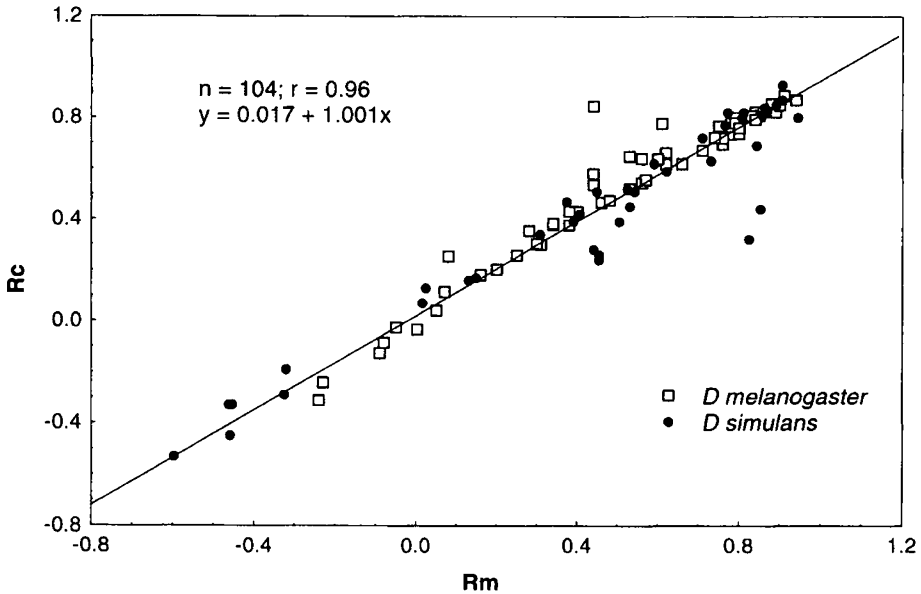
Figure 3. Comparison of the two estimates of the genetic correlation. r_m: correlation calculated directly from family means; r_c: correlation corrected for family size (see text for details).

across species, populations and temperatures (Spearman rank correlation $r_S = 0.87$, $n = 126$, $P < 0.0001$). Phenotypic correlations were biased downwards, however. The average difference between the two correlations was 0.14 (s.e. 0.02). In general, genetic correlations were greater than phenotypic correlations, except when all correlations tend to zero.

4. DISCUSSION AND CONCLUSIONS

4.1. An antero-posterior gradient

There was a progressive decrease of all correlations (phenotypic, genetic and environmental) as body segments became more distant (*figure 2*). For example, the pigmentation of the mesothorax was positively correlated with that of abdominal segment 5 in more cases than with that of abdominal segment 7. In previous work [9, 13] it was argued that different shapes of reaction norms in different segments demonstrated that their genetic bases were not exactly the same. The regular decrease in the genetic correlations with segmental distance is further proof of that conclusion. Investigating correlations between values of the same trait in different environments is central in understanding the genetics of phenotypic plasticity [10, 37, 38]. Falconer [11] proposed that the same trait measured in two environments could be considered as two different traits related by their genetic correlation.

Table II. Coefficients of correlation calculated among family means, without correction (r_m) for each temperature. Each value is based on ten observations. Threshold values: 0.63 for $P = 0.05$, 0.76 for $P = 0.01$ and 0.87 for $P = 0.001$.

	5–6	5–7	6–7	5–trid	6–trid	7–trid
			D. melanogaster Bordeaux			
14 °C	0.91	0.56	0.76	0.38	0.40	−0.05
17 °C	0.85	0.71	0.94	0.30	0.16	0.34
21 °C	0.84	0.78	0.85	0.05	−0.23	−0.08
25 °C	0.84	0.62	0.79	0.44	0.002	−0.09
28 °C	0.66	0.31	0.79	0.61	0.28	0.07
31 °C	0.62	0.55	0.71	0.44	−0.24	0.02
Mean ± s.e.	0.79 ± 0.04	0.59 ± 0.06	0.81 ± 0.03	0.37 ± 0.07	0.06 ± 0.10	0.03 ± 0.06
			D. melanogaster Villeurbanne			
14 °C	0.89	0.76	0.89	0.80	0.86	0.74
17 °C	0.83	0.78	0.88	0.53	0.62	0.25
21 °C	0.84	0.79	0.90	0.46	0.60	0.38
25 °C	0.78	0.57	0.80	0.83	0.53	0.34
28 °C	0.80	0.44	0.68	0.56	0.20	0.08
31 °C	0.78	n.c.	n.c.	0.75	0.48	n.c.
Mean ± s.e.	0.82 ± 0.02	0.67 ± 0.06	0.83 ± 0.04	0.65 ± 0.06	0.55 ± 0.08	0.36 ± 0.10
			D. simulans Bordeaux			
14 °C	0.47	0.34	0.82	0.16	−0.29	−0.53
17 °C	0.80	0.59	0.81	−0.33	−0.33	−0.45
21 °C	0.83	0.63	0.80	−0.09	0.04	−0.05
25 °C	0.50	0.25	0.72	n.c.	n.c.	n.c.
28 °C	0.93	0.89	0.87	0.53	0.57	0.54
31 °C	0.84	0.26	0.24	−0.09	0.10	−0.59
Mean ± s.e.	0.73 ± 0.07	0.49 ± 0.09	0.71 ± 0.09	0.04 ± 0.13	0.02 ± 0.14	−0.22 ± 0.19
			D. simulans Villeurbanne			
14 °C	0.85	n.c.	n.c.	0.45	0.39	n.c.
17 °C	0.62	0.52	0.77	−0.54	−0.51	−0.57
21 °C	0.13	0.07	0.87	n.c.	n.c.	n.c.
25 °C	0.42	0.17	0.82	n.c.	n.c.	n.c.
28 °C	0.51	−0.19	0.39	n.c	n.c.	n.c.
31 °C	0.51	0.28	0.69	0.45	0.44	0.32
Mean ± s.e.	0.51 ± 0.09	0.17 ± 0.10	0.71 ± 0.08	0.12 ± 0.27	0.11 ± 0.25	−0.12 ± 0.31

n.c.: not calculated because of a null variance in one trait. The six possible pairwise correlations between segments are indicated as follow: 5, 6 and 7: abdomen segments 5, 6 and 7; trid: thoracic trident (mesonotum).

David et al. [9] showed that when pigmentation scores of the same lines at different temperatures were compared, the correlation decreased regularly when more distant temperatures were considered. This phenomenon was also observed in the present work (results not shown). Here we demonstrated that this pattern also holds for correlations between different segments, at a given temperature.

Table III. Average values ($\pm$ s.c.) of the within-line correlation. Coefficients were calculated separately within each sample of ten females of the same line grown at the same temperature. Values were then averaged over temperatures to produce a line value.

Trait	5–6	5–7	6–7	5–trid	6–trid	7–trid
D. melanogaster Bordeaux	0.44 ± 0.05 (10)	0.27 ± 0.06 (10)	0.51 ± 0.07 (10)	−0.02 ± 0.04 (10)	0.003 ± 0.02 (10)	−0.02 ± 0.07 (10)
D. melanogaster Villeurbanne	0.41 ± 0.04 (10)	0.30 ± 0.06 (10)	0.52 ± 0.04 (10)	0.28 ± 0.05 (10)	0.12 ± 0.06 (10)	0.12 ± 0.07 (10)
D. simulans Bordeaux	0.37 ± 0.02 (10)	0.24 ± 0.05 (10)	0.44 ± 0.06 (10)	0.21 ± 0.05 (10)	0.09 ± 0.05 (10)	0.03 ± 0.07 (10)
D. simulans Villeurbanne	0.44 ± 0.04 (10)	0.25 ± 0.06 (10)	0.34 ± 0.07 (10)	0.10 ± 0.09 (8)	0.01 ± 0.09 (8)	0.04 ± 0.10 (5)

( ): Number of lines used in calculation. These line values, in most cases ten in each population, were used to calculate the average correlations in the table.

Pigmentation genes are expressed differently across environments and body segments. The fact that pigmentation expression varies according both to body segment and temperature indicates the existence of a complex interaction between internal and external environments. But this interaction has regularities. The embryonic determinism of body segments and the role of homeotic genes are now well known in *Drosophila* [21]. Our knowledge on the development of the epidermis of adult segments is, however, much less [12] so that any precise hypothesis on the nature of interacting genes seems premature.

4.2. Phenotypic correlations and Cheverud's conjecture

For the analysis of natural populations, various fitness-related traits are often measured on different individuals, providing an estimate of phenotypic correlation. Evolutionary theory has emphasised the major significance of trade-offs between characters for explaining the stability of life history strategies [4, 28, 34, 35]. Since evolution is dealing with genetic variations, estimating heritabilities and genetic correlations in natural populations appears as a major issue in evolutionary biology. In various investigations, both phenotypic and genetic correlations have been measured, and in many cases similar values have been observed [5, 29]. This led Cheverud to propose his conjecture according to which phenotypic correlations could be used in many cases as a crude estimate of genetic correlations.

The isofemale line technique provides an estimate of the genetic variability of any trait [3, 17]. When two traits are measured on the same individual, the between-line covariance also allows an estimate of the genetic correlation. Except when family size is sufficiently large ($n > 20$) the variance and covariance between families should be corrected by the within family terms. In the present work, a total of 144 values of r_g could be calculated, and it was thus possible to test empirically the efficiency of the correction. Quite surprisingly, both methods provided very similar coefficients of correlation. Additionally, in about 17 % of cases, the corrected values could not be calculated. Practically, and at least when family number is quite small, it seems better to estimate the genetic correlation by simply using the family means, as already suggested by Via [37].

We obtained confirmation but also invalidation of Cheverud's [5] conjecture. On the one hand, we found that the two sets of correlations were highly correlated and varied in a parallel way. On the other hand, the genetic correlations were regularly and consistently greater that the phenotypic ones. In our experimental protocol, we kept the environmental variance and covariance (as estimated by the within-line values) as small as possible through the control of the experimental conditions: constant temperature and good larval feeding. This might explain the low value of the within-line correlations. It is, thus, unclear to what extent that Cheverud's conjecture might better apply to natural populations living in a variable environment, or to endothermic species.

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