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Variation in genetic architecture of olfactory behaviour among wild-derived populations of *Drosophila melanogaster*

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Abstract

Odour-guided behaviour is a quantitative trait determined by many genes that are sensitive to gene–environment interactions. Different natural populations are likely to experience different selection pressures on the genetic underpinnings of chemosensory behaviour. However, few studies have reported comparisons of the quantitative genetic basis of olfactory behaviour in geographically distinct populations. We generated isofemale lines of *Drosophila melanogaster* from six populations in Argentina and measured larval and adult responses to benzaldehyde. There was significant variation within populations for both larval and adult olfactory behaviour and a significant genotype × sex interaction (GSI) for adult olfactory behaviour. However, there is substantial variation in the contribution of GSI to the total phenotypic variance among populations. Estimates of evolvability are orders of magnitude higher for larvae than for adults. Our results suggest that the potential for evolutionary adaptation to the chemosensory environment is greater at the larval feeding stage than at the adult reproductive stage.

Introduction

Darwin (1859) stated in 'The Origin of Species' that adaptive evolution is the result of the interaction between organisms and their environments. Because survival and reproductive success depend on environmental cues (Dominy *et al.*, 2004), traits that mediate perception of the environment can be targets for natural selection (Boake, 1994; Ortiz-Barrientos *et al.*, 2004). Many organisms assess the quality of their environments through chemical cues. Thus, chemosensory behaviour is critical for food localization, avoidance of environmental toxins or predators, interactions with reproductive partners and, for insects, localization of oviposition sites.

Odour-guided behaviour is a quantitative trait, determined by ensembles of multiple segregating genes that are sensitive to the environment (Fedorowicz *et al.*, 1998; Anholt *et al.*, 2001, 2003; Anholt, 2004; Anholt & Mackay, 2004; Sambandan *et al.*, 2006). *Drosophila melanogaster* presents a powerful model for dissecting the genetic underpinnings of olfactory behaviour, because large numbers of genetically identical individuals can be reared readily under controlled environmental conditions. Furthermore, the olfactory system of *Drosophila* is one of the best characterized chemosensory systems (Hallem *et al.*, 2006; Keller & Vosshall, 2007; Root *et al.*, 2007; Vosshall & Stocker, 2007; Anholt, 2008).

Previous studies have demonstrated that genes implicated in olfactory behaviour form functional ensembles through either additive or nonlinear interactions (Fedorowicz *et al.*, 1998; Anholt *et al.*, 2001, 2003; Sambandan *et al.*, 2006). It is perhaps not surprising that these networks are dynamic and sensitive to changes in the environment (Sambandan *et al.*, 2006).

Most studies to date have examined the effects of mutations in co-isogenic laboratory strains of *D. melanogaster* and focused exclusively on the genetic architecture of adult chemosensory behaviour. To place the dynamics of genetic networks in an ecologically relevant context, it is important to compare phenotypic variation and its underlying genetic causes in natural populations. Furthermore, chemosensory functions are vastly different in larvae (the feeding stage) and adults

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(the reproductive stage). Although their chemosensory system is remarkably simple, consisting of only 21 olfactory neurons (Stocker *et al.*, 1997; Tissot *et al.*, 1998; Gendre *et al.*, 2004; Fishilevich *et al.*, 2005; Gerber & Stocker, 2007; Keller & Vosshall, 2007), larvae respond to a large variety of chemicals (Ayyub *et al.*, 1990; Cobb *et al.*, 1992; Cobb & Dannet, 1994; Cobb, 1999; Cobb & Domain, 2000; Oppliger *et al.*, 2000).

Despite the wealth of knowledge about the molecular basis and physiological mechanisms of olfaction, natural phenotypic and genetic variation remain almost unexplored (but see Mackay *et al.*, 1996; Fanara *et al.*, 2002; Sánchez-Gracia *et al.*, 2003). Furthermore, it is likely that the genetic architecture that underlies larval olfactory behaviour is distinct from that in the adult. Moreover, different selection forces that drive adaptations are likely to act on larval survival and on adult survival and reproduction.

Phenotypic plasticity and genotype × environment interaction (GEI) are two genetic processes that can lead to adaptation. Phenotypic plasticity (i.e. environmental sensitivity) is the property of a genotype to produce different phenotypes in response to distinct environments (Schmalhausen, 1949; West-Eberhard, 1989; Schlichting & Pigliucci, 1998; Conner & Hartl, 2004; Fordyce, 2006; Mackay & Anholt, 2007). It plays a major role as a diversifying factor in evolution contributing to the origin of novel traits and altered directions of changes (West-Eberhard, 1989). A plot of the mean phenotypic value of a genotype across a range of environments is its reaction norm. However, not all genotypes respond the same way to environmental changes; variation in the reaction norm is manifested as GEI. The study of phenotypic plasticity and GEI is complex because it requires the understanding of its underlying genetic variation, its liability to environmental conditions and its association with fitness (Via *et al.*, 1995; Schlichting & Pigliucci, 1998; Debat & David, 2001). Genetic variation can be maintained in the face of environmental heterogeneity by GEI (Via & Lande, 1987; Gillespie & Turelli, 1989; Gurganus *et al.*, 1998; Ungerer *et al.*, 2003). This is specially important in the case of genotype × sex interaction (GSI, which is a special case of GEI, where sex defines the environment in which the genome operates) as it has previously been hypothesized that this particular case of GEI contributes to the maintenance of genetic variation (Mackay *et al.*, 1996; Mackay, 2001, 2004; Fanara *et al.*, 2002; Anholt *et al.*, 2003; Harbison *et al.*, 2004).

To assess variation in behaviour and GSI among different natural populations that occur in different environments, we have measured olfactory behaviour for both larvae and adults from multiple isofemale lines derived from six natural populations of *D. melanogaster* in Argentina. These populations represent a geographically distinct latitudinal cline, which differ in altitude and host plant availability. We show that there is substantial variation in olfactory behaviour within each population. However, there is also considerable variation in olfactory responsiveness between populations, indicative of genetic differentiation between populations. Furthermore, the observed genetic variation shows sexual dimorphism in adult flies and GSI that varies among populations. Finally, our results indicate that some aspects of the genetic architecture for olfactory behaviour differ between larvae and adults. Thus, this collection of wild-derived *Drosophila* lines from the six Argentinian populations provides a resource for a systematic analysis of the genetic underpinnings of behavioural plasticity and GEI.

Materials and methods

Drosophila stocks

Gravid *D. melanogaster* females were collected from six natural populations from Argentina (Neuquén, Uspallata, Lavalle, Guemes, Chilecito and Cachi) and used to found isofemale lines (David *et al.*, 2005). Geographical location, host plant prevalence, latitude, longitude, altitude and climatological information for each population are presented in Fig. 1 and Table 1. All lines were maintained by full-sib mating for 10 generations on cornmeal–molasses–agar medium under standard conditions of 25 ± 1 °C, 70% humidity and a 12-h light : 12-h dark cycle; lights were switched on at 08:00 hours and switched off at 20:00 hours. Ten to 12 lines from each population were randomly chosen for the analysis of larval and adult odour-guided behaviour.

Behavioural assays

Olfactory behaviour was measured for both larvae and adults using benzaldehyde as a standard odorant. To measure larval olfactory behaviour, we used the assay of Aceves-Piña & Quinn (1979), modified by Cobb *et al.* (1992). Adult females were allowed to lay eggs for 8 h on agar medium with yeast paste. Larvae were tested after 36 h and washed from the yeast paste prior to testing. Between 10 and 30 larvae were placed at the centre of a 10-cm Petri dish containing 10 ml of 2.5% agar. A 5-μl drop of 1% (v/v) benzaldehyde (Merck Schuchardt OHG, Hohenbrunn, Germany), which is an attractant for larvae (Ayyub *et al.*, 1990; Cobb *et al.*, 1992; Oppliger *et al.*, 2000; Ganguly *et al.*, 2003), and a 5-μl drop of distilled water were placed on filter paper discs on opposite ends of the Petri dish. To prevent diffusion of odorants through the agar and to eliminate larval gustatory responses, the filter paper discs containing the odorant or water were placed on inverted lids cut off 1.5 ml microcentrifuge tubes. Five minutes after the introduction of the larvae, the number of individuals within a 30-mm radius from each filter disc and the larvae that remain between both 30-mm radii were counted. Olfactory responses tend to decline after 5 min, presumably as

Fig. 1 Locations of the six natural populations from Argentina: 1 = Guemes (Province of Salta), 2 = Cachi (Province of Salta), 3 = Chilecito (Province of La Rioja), 4 = Lavalle (Province of Mendoza), 5 = Uspallata (Province of Mendoza) and 6 = Neuquén (Province of Neuquén). Geographical coordinates for these populations are given in Table 1.

a result of saturation of the vapour phase (Rodrigues, 1980). A larval response index (LRI) was calculated for each dish as:

$$\mathrm{LRI} = [(n_{\mathrm{odorant}} - n_{\mathrm{control}})/n_{\mathrm{total}}] \times 100$$

where n designates the number of larvae and the subscripts indicate the sides of the Petri dish containing odorant or water respectively. This index varies between −100 (total repulsion) and +100 (total attraction). An RI = 0 indicates indifferent behaviour. When tested in groups, individual larvae respond to odorants independent of the other individuals (Monte *et al.*, 1989). All behavioural tests were performed between 14:00 and 16:00 hours under controlled temperature (25 ± 1 °C), light (5.4 ± 0.2 × 10^5 lx) and humidity (42 ± 5%).

To measure odour-guided behaviour of adult flies, we used the assay described by Anholt *et al.* (1996). Flies were collected 3–5 days after eclosion using light CO_2 as anaesthetic, and were stored in single-sex groups of five individuals in 2.5 × 8-cm plastic vials without medium. The experimental assay was performed 2 h later to allow flies to recover from the effect of CO_2. Vials were marked with two lines, 3 and 6 cm from the bottom. A cotton swab dipped in a discriminating submaximal concentration of 0.3% (v/v) benzaldehyde was inserted so that the tip of the cotton swab lined up against the 6-cm mark. The vial was placed horizontally during the assay with both sides of the vial closed with white coloured surfaces to avoid the effect of negative geotaxis and phototaxis respectively. After flies were allowed to recover for 15 s from the disturbance of the insertion of the cotton swab, 10 counts of the number of flies in the bottom 3 cm of the vial were taken at 5 s intervals. The adult response index (ARI) was calculated for each sex as the average of the 10 measurements, and varies between 0 (total attraction) and 5 (total repulsion). All behavioural tests were performed between 14:00 and 16:00 hours under controlled temperature (25 ± 1 °C), light (1.18 ± 0.02 × 10^6 lx) and humidity (42 ± 8%). Five to seven replicate measurements were made for males, females and larvae of each line.

Statistical analyses

We used an analysis of variance (ANOVA) to evaluate the sources of LRI variance in larval olfactory behaviour among populations (P) according to the mixed model: $Y = \mu + P + L(P) + E$, where P is a fixed effect; L the random effect of line, E the error variance and parentheses indicate nested effects. To analyse variation in adult olfactory behaviour, we used a three-way nested mixed ANOVA model: $Y = \mu + P + S + L(P) + P \times S + L(P) \times S + E$, where S is the fixed effect of sex. A significant L

Table 1 Characteristics of six populations of *Drosophila melanogaster* from Argentina.

Population	Host	Latitude (south)	Longitude (west)	Altitude (m)	Mean annual temperature (°C)	Number of lines analysed Larvae	Adults
Guemes	Unknown	24°41′	65°03′	695	20.2	11	10
Cachi	Grapes	25°07′	69°09′	2280	10	10	10
Chilecito	Grapes	29°10′	67°28′	1043	20	11	12
Lavalle	Grapes, quince	32°50′	68°28′	647	17.1	10	10
Uspallata	Apple, quince	32°35′	69°22′	1915	12.2	10	10
Neuquén	Apple	38°57′	68°04′	260	–*	10	11

*No datum is available for Neuquén.

effect indicates genetic differences among lines for the traits analysed, whereas a significant $L(P) \times S$ interaction is interpreted as an estimate of GSI. Significant GSI can arise from: (1) differences in the among-line variance in males and females (change in magnitude); and/or (2) deviations from unity of the cross-sex genetic correlation ($r_{GSI} < 1$; changes in rank order). We analysed the contribution of these two sources of variation using the equation derived by Robertson (1959),

$$\sigma^2_{GSI} = [(\sigma_{males} - \sigma_{females})^2 + 2\sigma_{males}\sigma_{females}(1 - r_{GSI})]/2,$$

where σ^2_{GSI} is the GSI variance component, r_{GSI} is the cross-sex genetic correlation and, σ_{males} and $\sigma_{females}$ are the square roots of the among-line variance components of males and females respectively. The first term corresponds to differences in among-line variance of the two sexes, whereas the second term corresponds to deviations from the perfect correlation across sexes. The cross-sex genetic correlation ($r_{GSI} < 1$) is the genetic correlation of measurements in males and females and here reflects the degree to which the same genes control the phenotypic value in the two sexes. r_{GSI} was estimated as

$$r_{GSI} = \mathrm{cov}_{males\ females}/\sigma_{males}\sigma_{females},$$

where $\mathrm{cov}_{males\ females}$ is the covariance of olfactory behaviour between males and females.

All statistical analyses were performed using the GLM procedure implemented in the STATISTICA 6.0 software package (StatSoft, 2001).

Estimation of quantitative genetic parameters

Quantitative genetic parameters for olfactory behaviour were calculated for larvae and adults in each population. Assuming an additive model, the variance component among lines (σ^2_L) is an estimate of $2FV_G$, and the L × S variance component ($\sigma^2_{L\times S}$) is an estimate of $2F(\frac{1}{2}V_G)$, where F is the inbreeding coefficient (Mackay *et al.*, 1996). Due to half-sib mating during the establishment of the lines, for isofemale lines $F = 1/4$ (Hoffmann & Parsons, 1988); so, the genetic variance (V_G) for adult odour-guided behaviour equals $2\sigma^2_L + (\frac{1}{4})\sigma^2_{L\times S}$; the environmental variance (V_E) was estimated from the error variance, and the total phenotypic variance (V_P) was estimated as $V_G + V_E$. For larval olfactory behaviour, V_G is estimated as $2\sigma^2_L$,. V_E is the error variance and total phenotypic variance is the sum of V_G and V_E. Heritability (h^2) of both larval olfactory behaviour and adult olfactory behaviour across populations is estimated as $h^2 = V_G/V_P$ (Falconer & Mackay, 1996). We compared quantitative genetic properties of larval and adult olfactory behaviour in each population using the coefficient of genetic variance [$CV_G = 100(V_G)^{1/2}/X^2$, where V_G denotes genetic variance and X the population mean] as an estimate of evolvability and coefficients of environmental variance [$CV_E = 100(V_E)^{1/2}/X^2$]. These alternative measures are based on the notion that using trait mean values rather than variances is more appropriate for standardizing genetic variances for comparisons between different characters (Houle, 1992). Variance components were estimated with the Statistica Proc VARCOMP program.

Results

Phenotypic variation in olfactory behaviour of adult flies and larvae from different populations

We measured chemosensory responses to benzaldehyde of both larvae and adults from several isofemale lines derived from each of six Argentinean populations (Fig. 2). ANOVA revealed significant variation among lines for both larvae and adults (Table 2). However, whereas larval response indices for benzaldehyde showed extensive variation among populations, the population term in the ANOVA for adults was not significant. Indeed, mean values for olfactory responses to benzaldehyde were similar among adult flies from the different popu-

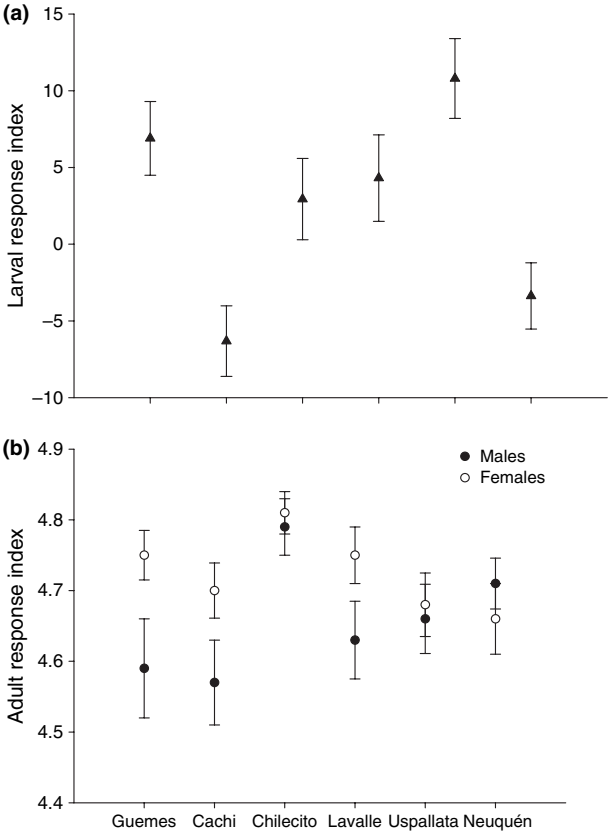

Fig. 2 Olfactory responses for larvae (a) and adults (b) of *Drosophila melanogaster* from different populations in Argentina. The means and standard errors of the response indices for benzaldehyde are presented. ▲ represents larvae mean RI, ● represents male adult mean RI and ○ represents female adult mean RI.

Table 2 Analysis of variance for larval and adult olfactory responses to benzaldehyde.

	Larvae			Adult		
Source	d.f.	MS	*P*	d.f.	MS	*P*
Population	5	3103.92	0.0019	5	0.47	0.503
Sex	–	–	–	1	0.95	0.0415
Population × sex	–	–	–	5	0.21	0.4483
Line (population)	56	723.07	0.0002	58	0.56	0.0003
Sex × line (population)	–	–	–	58	0.22	< 0.0001
Error	346	376.31	–	720	0.11	–

d.f., degrees of freedom; MS, mean squares.

lations, although flies from the Chilecito population tended to show slightly greater avoidance responses to benzaldehyde (Fig. 2). In addition to the greater variation in mean response values of larvae compared with that of adults, larvae from some populations (Neuquén and Cachi) showed avoidance responses, whereas larvae from all other populations showed attractant behaviour, with the highest mean response index observed with lines derived from the Uspallata population (Fig. 2). By contrast, adults from all populations showed only strong avoidance behaviour. We did not find a significant relationship between larvae or adult olfactory behaviour and latitude or altitude of origin ($F_{2,60} = 0.34$, $P = 0.71$ for latitude and altitude in adult male olfactory behaviour; $F_{2,60} = 0.53$, $P = 0.59$ for altitude in adult female olfactory behaviour; $F_{2,59} = 0.3$, $P = 0.74$ for latitude and altitude in larval olfactory behaviour).

A nonparametric Kruskal–Wallis ANOVA test showed statistically significant differences between the coefficients of variation (CV = standard deviation/mean) of larval and adult response scores within and across populations (Table 3). Thus, this could be indicative of substantial differences between larval and adult genetic architecture for olfactory behaviour.

Genotype × sex interaction effects

As reported previously, adult olfactory behaviour showed significant sexual dimorphism, indicated by a significant sex term in the ANOVA (Table 2). The ANOVA also showed significant effects by line and line × sex interaction across

Table 3 Nonparametric Kruskal–Wallis ANOVA test between the coefficients of variation of larval and adult response scores within and across populations.

	H	*P*
Uspallata	14.29	< 0.001
Guemes	11.88	< 0.001
Lavalle	14.29	< 0.001
Cachi	10.08	0.015
Neuquén	8.75	0.031
Chilecito	15.51	0.001
Across populations	76.38	< 0.001

Table 4 Phenotypic plasticity and relative contributions of sources of variation to the total phenotypic variation for adult and larval olfactory responses.

	Guemes	Cachi	Chilecito	Lavalle	Uspallata	Neuquén
Adult						
Line	45.6*	28.4*	8.4*	0	8.4*	0.8
Line × sex	17.9***	2.9	0	41.8*	0	5.6
Larvae						
Line	15**	20**	11.2**	15**	0	14.4*

Numbers are percentages of total phenotypic variation.
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

populations (Table 2). However, the population × sex term was not significant, indicating that either the GSI effect *per se* does not vary among populations or the number of populations surveyed is not sufficiently large to resolve GSI differences between them (Table 2). Whereas GSI did not contribute to overall phenotypic variation in the Uspallata, Chilecito, Neuquén and Cachi populations, GSI accounted for 17.9% and 41.8% of the phenotypic variation in the Guemes and Lavalle populations respectively (Table 4).

To further investigate the nature of GSI in the Guemes and Lavalle populations, we constructed reaction norms for olfactory responses of males and females for 11 lines from Guemes and 10 lines from Lavalle (Fig. 3). In each case, males showed greater variation in mean avoidance responses across lines than females. Furthermore, the observed deviation from parallelism of reaction norms is diagnostic of GSI. In the Guemes population, differences in rank order account for 7% of the GSI, whereas differences in response magnitude account for 93% of the GSI. In the Lavalle population, 22% of GSI is due to changes in magnitude, whereas 78% can be accounted for by change in rank order. Thus, the nature of GSI differs between these two populations.

Quantitative genetic analyses of olfactory behaviour of adult flies and larvae from different populations

To gain insights into the differences in genetic architecture of chemosensory responses in the different populations and between different stages of the life cycle, we calculated quantitative genetic parameters (Table 5). Estimates for genetic variance (V_G) were low for all populations, in agreement with estimates for V_G for adult olfactory behaviour of chromosome substitution lines in which second and third chromosomes extracted from a natural *D. melanogaster* population from North America were introgressed in a common co-isogenic background (Mackay *et al.*, 1996). It is of interest to note that the value for V_G for larvae for the Uspallata population is zero and for adults close to zero. This is a high-altitude, isolated population (Table 1), which may have experienced inbreeding after a bottleneck phase resulting in loss of genetic variation.

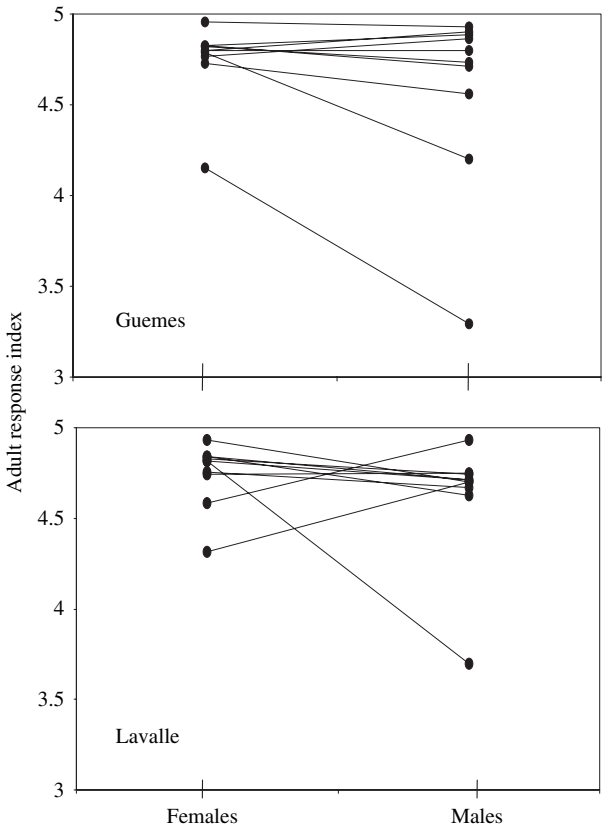

Fig. 3 Norms of reaction for adult response index for benzaldehyde in the two sexes for 10 isofemale lines from the Guemes (top panel) and Lavalle (bottom panel) populations.

Table 5 Estimates of quantitative genetic parameters of olfactory responses.

	Guemes	Cachi	Chilecito	Lavalle	Uspallata	Neuquén
Adult						
V_G	0.2	0.1	0.02	0.32	0.02	4.10^{-3}
CV_G	2.08	1.47	0.57	2.59	0.72	0.3
V_E	0.08	0.12	0.1	18.43	0.14	0.14
V_P	0.28	0.22	0.1	19.25	0.14	0.15
h^2	0.72	0.46	0.17	0.02	0.17	0.03
Larvae						
V_G	131.19	150.63	117.74	142.96	0	90.7
CV_G	22.55	30.82	168.52	64.37	0	83.86
V_E	370.85	302.09	473.7	403.73	436.52	270.44
V_P	502.04	452.72	591.44	546.69	436.52	361.14
h^2	0.26	0.33	0.12	0.26	0	0.25

V_G, V_E and V_P indicate the genetic, environmental and phenotypic variances respectively; CV_G and h^2 indicate coefficients of genetic variance and heritability, respectively.

Heritability (h^2) estimates are generally low for both larvae and adults, as is expected for fitness-related traits. Heritability estimates for larvae and adults of the Uspallata population are virtually zero, due to the absence of genetic variation. Whereas there is general concordance between larval and adult heritability estimates, heritability of olfactory avoidance response for adult flies from the Guemes population is unusually high ($h^2 = 0.723$), because of the relatively low environmental variance (V_E) in this population compared with V_G (Table 5). The host plant of the *D. melanogaster* population from Guemes is not known (Table 1).

Estimates of evolvability using CV_G for larvae and adults from different populations ranged from 0 to 168.52 and from 0.3 to 2.59 respectively (Table 5). Strikingly, evolvability estimates for larvae were dramatically higher than that for adults, generally by orders of magnitude, in each population (Table 5). Together with the substantial variation in larval compared with adult olfactory responses in the different populations, described in Fig. 2, these data suggest that the potential for evolutionary adaptation to the chemosensory environment is greater at the larval feeding stage than the adult reproductive stage.

Discussion

We conducted a comparative study of the genetic basis of phenotypic variation for olfactory behaviour in six Argentinean populations of *D. melanogaster* using wild-derived isofemale lines. Previous studies on chemosensory behaviour in different populations have focused exclusively on adult olfactory behaviour and have only characterized phenotypic variation (Alcorta & Rubio, 1989) or genetic variation from a single natural population (Mackay *et al.*, 1996; Wang *et al.*, 2007), but have not reported quantitative genetic parameters that underlie differences in phenotypic variation between populations. Here, we have shown that variation in larval olfactory behaviour exceeds the variation observed for adults in isofemale lines among populations and, in line with this observation, that evolvability estimates are consistently higher for larvae than for adults (Table 5). Further analyses should assess whether these differences are the results of different selective forces acting on larval and adult olfactory behaviour.

Except for the Guemes populations, heritability estimates were generally low, in concordance with estimates reported previously for co-isogenic substitution lines in which wild-derived *X* or third chromosomes were introgressed (Mackay *et al.*, 1996). Low heritability and large CV_G estimates are expected for fitness-related traits (Mousseau & Roff, 1987; Houle, 1992; Falconer & Mackay, 1996). We also found that genetic variance is greatly variable among populations, reflecting different population histories. In addition, the contribution of GSI to the total phenotypic variance varies widely between 0% and 42% (Table 4). The absence of GSI in the Chilecito and Uspallata populations is perhaps not surprising as both populations have

negligible genetic variance for adult olfactory response to benzaldehyde.

The absence of genetic variation for larval and adult olfactory behaviour in the Uspallata population probably reflects a history of inbreeding. The dichotomy of substantial genetic variation for larvae and near absence of genetic variation in adults for the response to benzaldehyde in the Chilecito population is puzzling, but would imply substantial contributions to the larval olfactory phenotype of a segment of the genome that contains elements that do not contribute to the genetic networks that underlie adult olfactory response to this odorant. Previous work have shown that the families of odorant-binding protein (*Obp*) genes and odorant receptor (*Or*) genes are thought to evolve rapidly (Young *et al.*, 2002; Robertson *et al.*, 2003) and that some of these genes are differentially expressed in larvae and adults (Hekmat-Scafe *et al.*, 2002; Fishilevich *et al.*, 2005). Furthermore, population genetic analyses of DNA sequences of 13 *Obp* genes in inbred lines derived from a Raleigh, NC, natural population have shown that different members of the odorant-binding protein gene family have different evolutionary histories (Wang *et al.*, 2007). It is possible that greater genetic variance for adult olfactory behaviour in the Chilecito population would be observed if behavioural responses were measured for other odorants.

What are the underlying causes of the difference observed between larval and adult variation in olfactory response? *A priori* temperature does not seem to be an important factor for geographic variation in olfactory behaviour, as it is for life-history traits (Partridge *et al.*, 1995) and for morphological traits (Barker & Krebs, 1995; Loeschcke *et al.*, 1999). Instead host fruit availability appears as a possible determining cause for the patterns of variation between populations for olfactory behaviour. Different chemical environments produced by the host fruits for larvae and adults (Kreher *et al.*, 2005) could result in different environmental pressures. We favour the latter hypothesis, as we did not find a latitudinal or altitudinal cline for adults or larvae (temperatures are vastly different along latitudinal and altitudinal gradients). Moreover, the differences between larval and adult quantitative genetic parameters suggest a greater susceptibility to environmental differences in larval olfactory behaviour, which could lead to local adaptation in larval olfactory behaviour. Because differences between larvae and adults could be due to differences in the assays used to measure olfactory behaviour, we performed comparisons between different stages of the life cycle by estimating coefficients of variation, i.e. CV_G.

Finally, one confounding factor is the unknown composition of the complex mixture of ecologically relevant odorants from the natural environments to which flies in the different populations adapt, e.g. in the Guemes population the host plant for *D. melanogaster* has not yet been identified (Table 1). Further studies would have to assess to what extent differences in variation and heritability for larval and adult olfactory responses, and GSI heterogeneity between the different populations are universal features for behaviours elicited by different odorants.

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