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Evolution of sexual dimorphism in the olfactory brain of Hawaiian *Drosophila*

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In the fruitfly, *Drosophila melanogaster*, mate choice during courtship depends on detecting olfactory cues, sex pheromones, which are initially processed in the antennal lobe (AL), a primary olfactory centre of the brain. However, no sexual differences in the structure of the AL have been found in *Drosophila*. We compared the central brain anatomy of 37 species of Drosophilidae from the islands of the Hawaiian archipelago, uncovering an extreme sexual dimorphism within the AL in which two out of the 51 identifiable glomeruli were markedly enlarged in males. A phylogeny indicated that the sexual dimorphism of the homologous glomeruli arose 0.4–1.9 Myr ago independently in two species groups of Hawaiian endemic Drosophilidae. The corresponding glomeruli in *D. melanogaster* were also found to be sexually dimorphic. The formation of glomeruli of male size is prevented by the ectopic expression of female-type *transformer* (*tra*) cDNA in males, indicating that the glomerular sexual dimorphism is under the control of the sex-determination cascade of genes. It is suggested that a defined set of glomeruli in *Drosophila* can enlarge in response to sex-determination genetic signals, the mutations of which may result in species differences in sexual dimorphism of the brain.

Keywords: antennal lobe; sexual dimorphism; Hawaiian Islands; gene expression; evolution; *Drosophila*

1. INTRODUCTION

Sexual dimorphism in the brain, with neurons unique to one sex or nuclei that differ in cell number between the sexes, has been recognized in a wide variety of both vertebrates (Tobet & Fox 1992; Hofman *et al.* 1993; Breedlove 1994) and invertebrates (Strausfeld 1980, 1991; Possidente & Murphey 1989; Taylor & Truman 1992). As in the mammalian olfactory bulb, the antennal lobe (AL) in insects is composed of a modular unit, the glomerulus (Schneiderman *et al.* 1982; Boeckh & Tolbert 1993; Galizia *et al.* 1999; Laissue *et al.* 1999). Some glomeruli in the males of moths (Schneiderman *et al.* 1986), cockroaches (Boeckh & Tolbert 1993) and bees (Galizia *et al.* 1999) are remarkably large in size, referred to as the male-specific macroglomeruli and are sporadically found in phylogenetically distant insect lineages. This suggests that the apparent similarity of the macroglomeruli across species is a result of functional convergence. Alternatively, the macroglomeruli in different species may originate from a common ancestral structure, which evolved only once, as implied by their consistent location. With the aim of resolving the issue of functional convergence versus evolutionary conservation in the formation of sexually dimorphic glomeruli, we conducted a systematic analysis of the AL structure in 37 species of Hawaiian endemic Drosophilidae. A very precise phylogenetic tree is available for this unique Drosophilid group, which was founded presumably by a single fertilized female that migrated to the Hawaiian Islands from the continent of Asia (Carson 1970, 1987, 1997). We succeeded in demonstrating that sexual dimorphism in the anatomically homologous glomeruli in the brain arose repetitively in different groups during evolution. In one species group, we deduced that the sexually dimorphic glomeruli were produced *ca.* 2 Myr ago on an ancient island of Maui–Lanai–Molokai island complex, 'Maui Nui'. We further demonstrated, using the fruitfly, *Drosophila melanogaster*, that the glomerular sexual dimorphism is under the control of the sex-determination cascade of genes, as the formation of glomeruli of male size is prevented by the ectopic expression of female-type *transformer* (*tra*) cDNA in males. We propose that a defined set of glomeruli can enlarge in response to the sex-determination signal.

2. MATERIAL AND METHODS

(a) *Flies*

Male and female Hawaiian *Drosophila* were collected from natural populations in Kauai, Molokai, Maui and Hawaii islands, or were supplied from laboratory stocks at the University of Hawaii and from the National *Drosophila* Species Resource Centre. *Drosophila* were maintained on a 12 L : 12 D cycle on standard cornmeal–yeast–agar medium at 25 °C.

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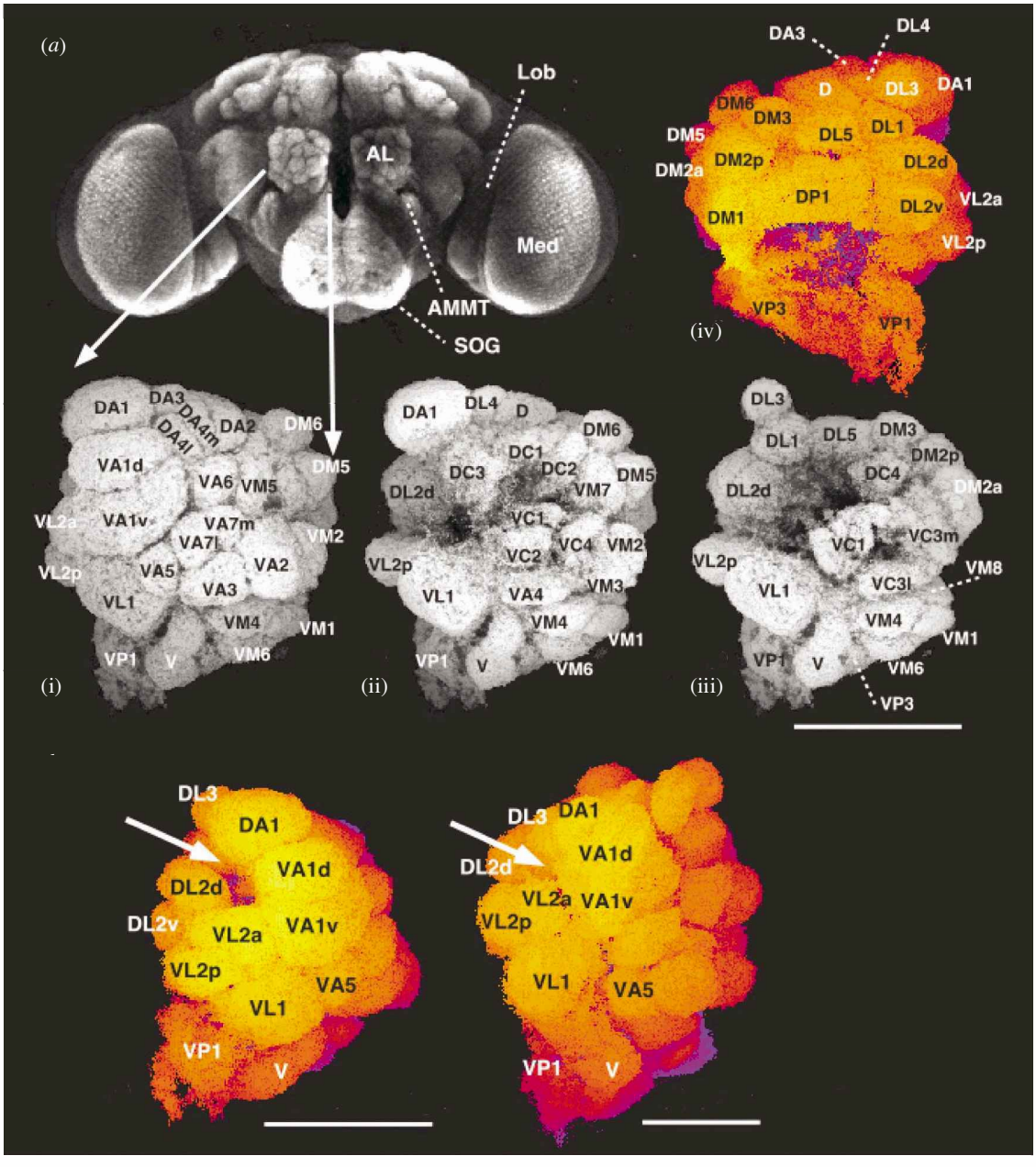

Figure 1. Virtual dissection of the AL in *Drosophila*, three-dimensional structures of which were reconstructed from the stacks of confocal images of glomeruli labelled with the anti-synaptotagmin antibody. (*a*) Projected views of the left AL in wild-type (Canton-S) *D. melanogaster*, viewed (i–iii) ventrally and (iv) dorsally. Although only the superficial glomeruli appear in the projected view (i, iv), internal glomeruli can be visualized individually by eliminating the images of the superficial glomeruli, as shown in (ii) and (iii). The nomenclature of each glomerulus within the AL is after Laissue *et al.* (1999). AL, antennal lobe; AMMT, antennal motor nerve and motor tract; Lob, lobular plate; Med, medulla; SOG, suboesophageal ganglion. Scale bar, 50 μm. (*b*) Comparison of the ALs in (i) female *D. melanogaster* and (ii) female *D. setosimentum*, viewed laterally. Rotation was −60° around the frontal–caudal axis (*y*-axis), and the projection parameters were set to no transparency. The arrows indicate the major insertion point of the primary antennal neurons into the AL. Scale bar, 50 μm.

(b) *Genetics and taxonomy*

69B-Gal4 (formally *P[w+, hs-GawB]69B*) and *Dll-Gal4* (*P[w+, hs-GawB]Dlmd23/CyO*) were obtained from the Bloomington *Drosophila* Stock Centre. *53B-Gal4* was a gift from J.-F. Ferveur of the Université de Bourgogne, and *esg-Gal4* (*yellow white; esg-Gal4[NP5130]*) was provided by S. Goto and S. Hayashi of the National Institute of Genetics of Japan. The *Gal4–UAS* (upstream activating sequence) system is described in Brand & Perrimon (1993).

The Hawaiian Drosophilidae can be classified into just two genera: *Drosophila* and *Scaptomyza* (Kaneshiro & Boake 1987). The genus *Drosophila* is further subdivided into four major groups: modified mouthparts; modified tarsus; fungus feeder; and picture wing, and several smaller groups including the *antopocerus* groups. The picture-wing group, a closely related complex of 103 species whose phylogenetic relationships are particularly well established based on chromosomal inversion (Carson 1970, 1987, 1997), comprises three major subgroups: the *adiastola* subgroup; the *planitibia* subgroup; and the *grimshawi* subgroup. Recent molecular analyses have revealed that the Hawaiian Drosophilids, including the genus *Scaptomyza*, are monophyletic despite their extreme morphological divergence

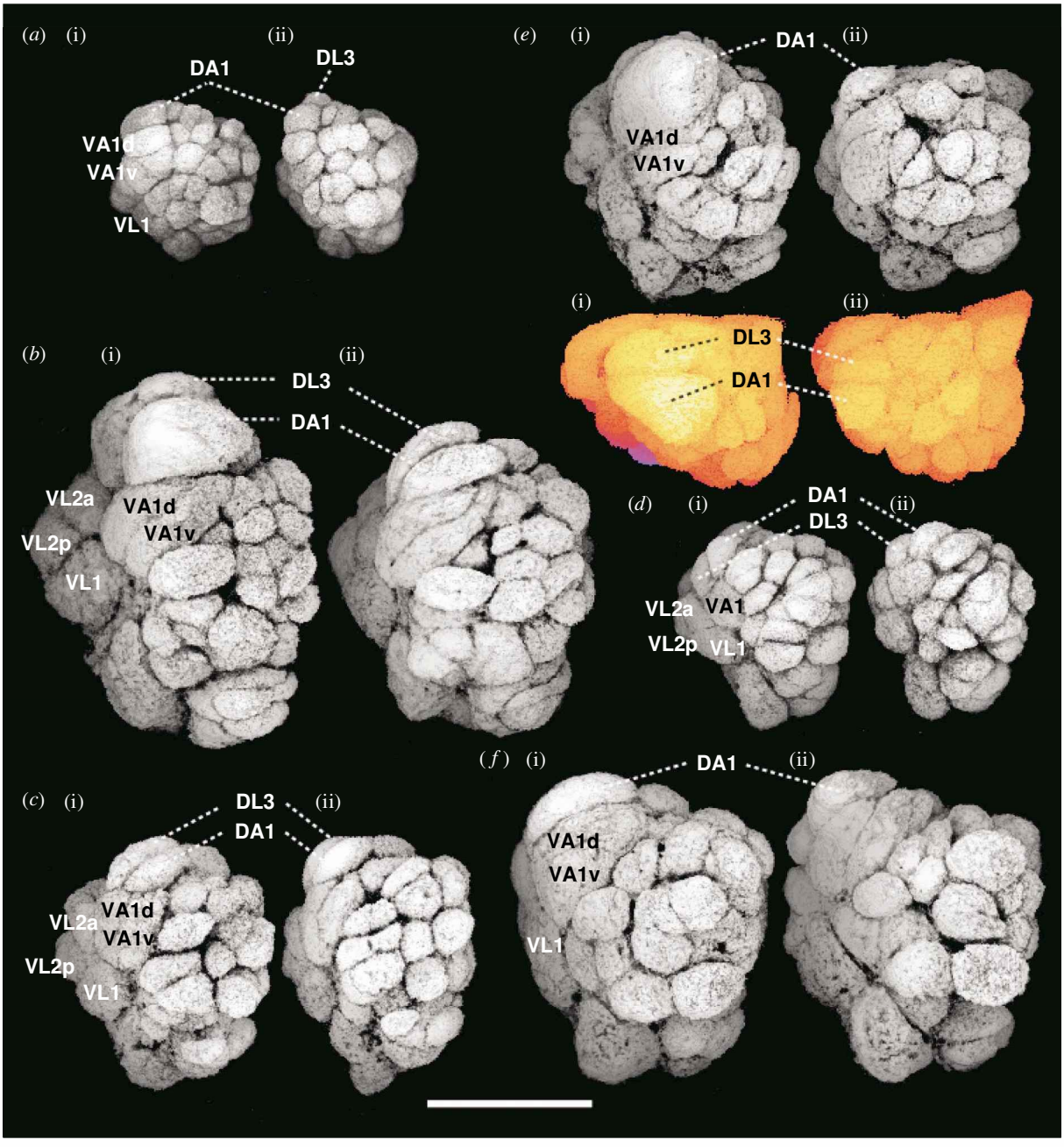

Figure 2. Three-dimensional structures of the AL. Ventral views of the AL from (i) male and (ii) female flies of wild-type (*a*) *D. melanogaster*; and Hawaiian *Drosophila* species, (*b*) *D. adunca*, (*c*) *D. diamphidiopoda*, (*d*) *D. mimica*, (*e*) *D. adiastola* and (*f*) *D. cyrtoloma*. The pseudo-colour images in (*e*) show the projected views of the male and female ALs from a −60° rotation around the horizontal axis, which was achieved by using the computer program NIH IMAGE v. 1.61. Scale bar, 50 μm.

(Davis *et al.* 2000). Systematically, the Hawaiian *Drosophila* species are more closely allied to the subgenus *Drosophila* than to the subgenus *Sophophola*, to which the model animal, *D. melanogaster*, belongs. However, there is no known single continental form that can be designated as the sister to the Hawaiian lineage.

The species belonging to the *adiastola* subgroup are very similar to the modified-mouthparts species in many respects. Similar to the modified-mouthparts species, the male labellum in *D. ornata*, the oldest member of the *adiastola* subgroup, is modified to have unusual hairs, bristles, spines and processes that are used for grasping the female's genital area during courtship (Hardy & Kaneshiro 1975).

(c) *Immunolabelling*

The glomerular architecture was visualized with an anti-synaptotagmin antibody (Littleton *et al.* 1993), and reconstructed using confocal microscopy and three-dimensional image processing. For immunolabelling of the glomerular structure, the proboscis was removed from the head capsule of the flies to expose the brain. The brain was fixed in 4% paraformaldehyde (with 3% Triton X-100 in 0.1 M phosphate buffer) for 1 hour at room temperature. After fixation, the brain was dissected out, washed in phosphate buffered saline containing 0.3% Triton X-100 (PBS-T) for 1 hour at 4 °C, and then blocked with 3% goat serum in PBS-T for 30 minutes. This was followed by an incubation in anti-synaptotagmin antibody (a gift from H. Bellen)

diluted 1 : 1000 in PBS-T for 2 days at 4 °C. After washing in PBS-T for 30 minutes, the brains were incubated in Cy3 conjugated anti-rabbit (Rhodamine red-X anti-rabbit IgG, Molecular Probes) diluted 1 : 1000 in PBS-T for 2 days at 4 °C. The brains were rinsed in PBS-T, and cleared in 50% glycerol to ensure minimum brain shrinkage.

Whole mounts of the brains were examined using a BioRad MRC 1024 confocal microscope. Images of individual ALs in the frontal, sagittal and horizontal planes were acquired at every 0.5–1 μm with 512 × 512 pixel resolution. The images were transferred to a Power Macintosh 8500/180 computer. To reconstruct the three-dimensional glomerular architecture, stacks of digitized images were processed by public-domain software, NIH Image v. 1.61 or Photoshop v. 5.0. The contour lines of the antennal glomeruli from each 0.5 μm optical section were traced manually and digitized on the computer screen by using a pen tablet (WACOM ArtZ II 12 × 12). The planimetric measurements were taken from the right AL wherever possible, with a few exceptions in which the left lobe was used. No significant left–right asymmetry was detected when the volumes of homologous glomeruli were compared in the two halves of a single brain. By using a macro-command of the NIH Image program, we counted the total number of pixels in the area enclosed by the contour lines, and spatially calibrated the image to allow precise area measurements. The images were rotated around the *x*-axis using the computer program NIH Image v. 1.61. Pseudo colour was added using the built-in colour look-up tables of the NIH Image program.

3. RESULTS AND DISCUSSION

The computer-aided three-dimensional image-processing technique, together with antibody labelling and confocal microscopy, allowed us to visualize the AL structure in detail (figure 1*a*). When neuropil regions in the brain of *D. melanogaster* were labelled with the anti-synaptotagmin antibody, as many as 51 glomeruli were recognizable in each AL of both male and female flies, including some that were previously unrecognizable (Laissue *et al.* 1999). Most of them are uniquely identifiable individually, based on their size and relative location within the AL neuropil (figure 1*a*).

Comparing the reconstructed images of the brains of Hawaiian Drosophilidae with the map of the *melanogaster* AL, we found that the basic plan of glomerular architecture is surprisingly similar across species (figure 2*b–f*). The arrangements of the major glomeruli in Hawaiian species are nearly identical to those in *D. melanogaster* (figures 1*b* and 2*a*). In the anterior lateral region of the AL there is the entry point of the antennal nerve—an excellent landmark for glomerulus identification. Around the entry point (shown by the arrows in figure 1*b*), there exist DA1, DL3, VA1d, VA1v (corresponding to VA1l and VA1m in Laissue *et al.* (1999)), VL1, VL2a, VL2p, DL2d and DL2v glomeruli in a Hawaiian *Drosophila, D. setosimentum*, just as in *D. melanogaster* (figure 1*b*).

Two glomeruli, the DA1 and DL3 glomeruli, lying side-by-side at the anterior lateral edge of the AL, were found to be sexually dimorphic in size in some Hawaiian Drosophilidae (figure 2*b*,*e*). For example, the volume of the DA1 glomerulus in *D. adunca* (figure 2*b*) was 3.4 times larger in males (mean ± s.e., $53.2 \pm 2.2 \times 10^3$ μm^3, $n = 12$) than in females ($15.5 \pm 1.1 \times 10^3$ μm^3, $n = 8$). In *D. adiastola* (figure 2*e*), the DA1 volume in males ($70.4 \pm 4.0 \times 10^3$ μm^3, $n = 6$) was 5.9 times larger than in females ($11.9 \pm 0.5 \times 10^3$ μm^3, $n = 10$). We observed such remarkable sexual dimorphism in only six out of the 37 species from the genus *Scaptomyza* and five groups of the genus *Drosophila*, all endemic to Hawaii (figure 2*b–f* and 3).

Importantly, these six species were found to belong to either the *antopocerus* group or the picture-wing group, and no species from other groups revealed any significant sexual dimorphism in glomerular organization (figure 3). Within the picture-wing group, the species possessing male-enlarged glomeruli are clustered in the *adiastola* subgroup, and none of the members of the remaining two subgroups, the *planitibia* subgroup (as shown in figure 2*f*) and the *grimshawi* subgroup, exhibited sexual dimorphism of the glomeruli. It should be emphasized that no evidence was obtained for glomerular sexual dimorphism in the species belonging to the modified-mouthpart group (for example, shown in figure 2*d*), which is phylogenetically intermediate between the *antopocerus* group and the *adiastola* subgroup. These observations support the hypothesis that sexually dimorphic glomeruli developed independently in two different species groups.

This hypothesis is further supported by the fact that, in the *adiastola* subgroup, only the descendant species exhibit marked sexual dimorphism of glomeruli, while the older members of the group do not (figure 4). Phylogenetic relationships, as determined by chromosomal inversion patterns, indicate that *D. setosimentum* is the newest species in the *adiastola* subgroup (Carson 1987). This species displays the most extreme sexual dimorphism in the volume of the DA1 glomerulus (figure 3*a*). The oldest member of the *adiastola* subgroup is *D. ornata*, which retains many characteristics common to the more primitive modified-mouthparts group, as presumably does the founder species of this subgroup (Carson 1987). In *D. ornata*, we found no sexual difference in the DA1 volumes (figure 4). The presumptive ancestral species, which must be sister species to *D. ornata*, is also the origin of two phylogenetic branches. One branch retains the modified mouthparts, while the other has lost them. In the former group, with modified mouthparts, we found only a marginal sexual dimorphism in the DA1 and DL3 glomeruli.

By contrast, while some of the species belonging to the *adiastola* subgroup, including *D. setosimentum*, have developed extreme differences in the volumes of these glomeruli between the two sexes, there are species within this subgroup that show only modest sexual dimorphism of the glomeruli. For example, *D. clavisetae*, a species belonging to a separate lineage from that of *D. spectabilis*, *D. adiastola*, *D. cilifera* and *D. setosimentum*, which displayed prominent sexual dimorphism of the glomeruli, shows only a moderate sexual dimorphism of the glomeruli. These facts suggest that the large expansion of the glomeruli in males has an origin in a putative ancestor common to *D. spectabilis*, *D. adiastola*, *D. cilifera* and *D. setosimentum*, after it diverged from another lineage, to which *D. clavisetae* and its close relatives belong (Carson 1987).

From the time of speciation to the present, the distribution of each Hawaiian endemic *Drosophila* species has been confined to narrow areas on an island owing to its low vagility, with rare founder events. Thus, the time of

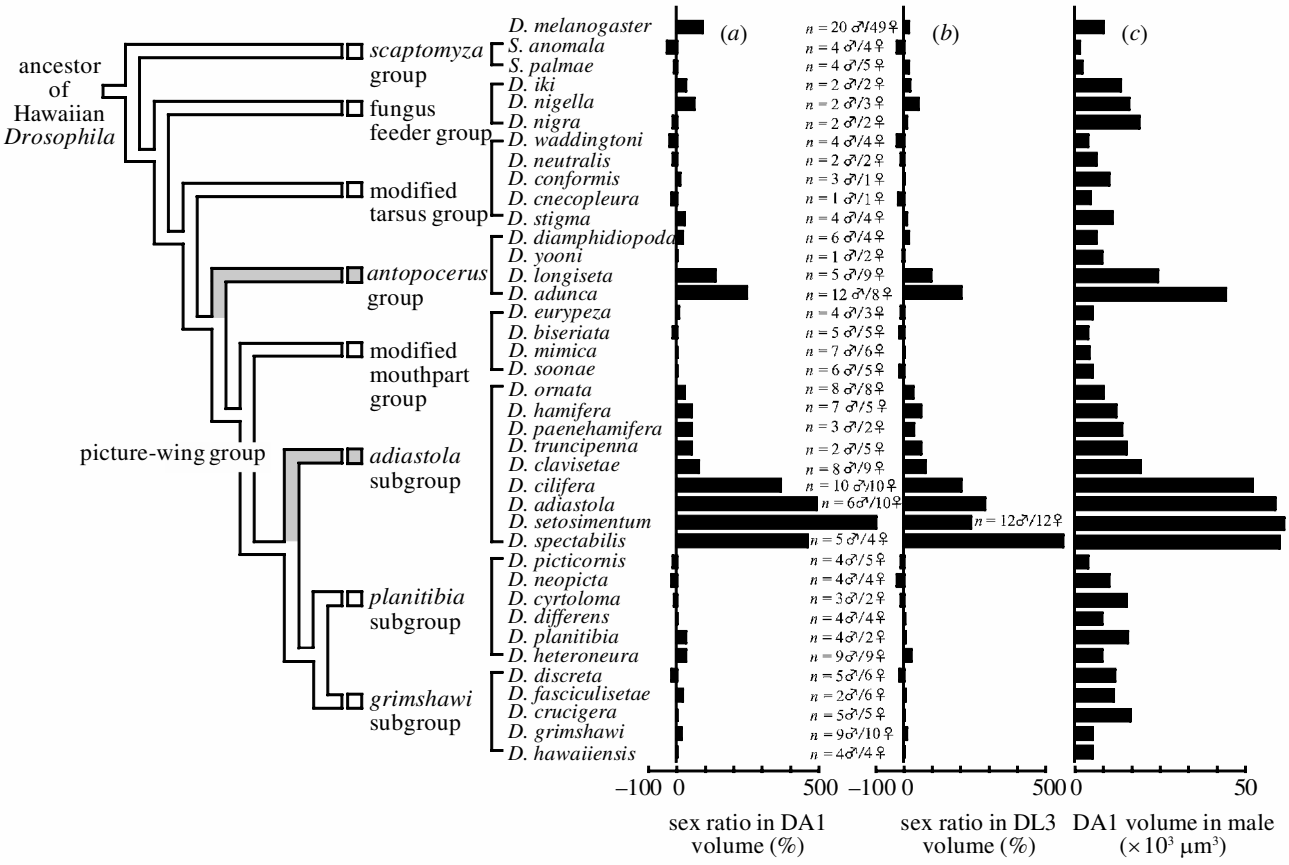

Figure 3. The glomerular size and its sexual dimorphism in two *Scaptomyza* species and 35 *Drosophila* species from Hawaii aligned in the order of their phylogenetic relationships. Phylogenetic relationships, based on chromosomal inversions and DNA sequences, are shown on the left. (*a*,*b*) Sexual dimorphism in the volumes of (*a*) DA1 and (*b*) DL3 as indicated by an index of [(male/female) – 1] × 100%. (*c*) The planimetric measurements of the DA1 volume in males, derived from confocal images digitized every 0.5 or 1 μm in depth. Bars represent the mean of values, and *n* is the number of male and female flies examined. These male-specific enlarged glomeruli have evolved independently in the *antopocerus* group and the picture-wing subgroup, which are placed on different branches of the phylogenetic tree. Shaded area, sexually dimorphic glomeruli present in some species, absent in other species; unshaded area, sexually dimorphic glomeruli absent.

emergence of each species can be deduced by comparing the geological history of the island that the species inhabits with the phylogenetic status of that species defined by chromosomal rearrangements and other biological characteristics (Carson 1970, 1987, 1997). The Hawaiian archipelago is a chain of islands *ca*. 700 km long from the oldest, Kauai (a potassium–argon age of 5 Myr), to the youngest, Hawaii (0.43 Myr; McDougall 1969). As a result of volcanic build-up about a fixed Hawaiian hot spot in the Earth's mantle and the northwestward movement of the Pacific tectonic plate over the hot spot, the islands successively emerged from the sea *ca*. every 1 Myr. Therefore, the original colonization of the ancient islands, *ca*. 24 Myr ago (McDougall 1969), was replayed island by island. This sequential colonization resulted in an explosive divergence of over 800 species, which represent more than one-quarter of all the known species of the family Drosophilidae in the world.

In the *adiastola* subgroup, *D. ornata* is the oldest species, with no glomerular sexual dimorphism, and, in fact, is the sole representative from the oldest island of Kauai. The main burst of the evolution of the *adiastola* subgroup occurred on the Maui–Lanai–Molokai island complex, Maui Nui. Three species with male-specific enlarged glomeruli, *D. adiastola* (east Maui), *D. cilifera* (Molokai) and *D. spectabilis* (Molokai), are all from this island complex. It is suggested that two species in Oahu, *D. touchardiae* and *D. neogrimshawi*, arose on Maui Nui and then back-migrated to Oahu. Therefore, it is plausible that the presumptive ancestor, which initially developed the huge glomeruli in the males, arose there. More precisely, this speciation event occurred at the time the ancient island of Maui Nui existed, *ca*. 1.9–0.4 Myr ago (McDougall 1969). After this period, less than 0.3–0.4 Myr ago, the large Maui Nui complex became two islands, one consisting of Molokai and Lanai and the second consisting of Maui and Kahoolawe. Less than 0.1–0.2 Myr ago, Kahoolawe separated from Maui, and, finally, Lanai separated from Molokai. *D. setosimentum*, the species with the largest male DA1 glomerulus, is the newest descendant to exhibit sexual dimorphism of the glomeruli, and evolved on the youngest island, Hawaii.

Encouraged by the discovery of a male-specific enlargement of the DA1 and DL3 glomeruli in several Hawaiian *Drosophila* species, we re-examined the AL of *D. melanogaster*, the sole *Drosophila* species amenable to detailed genetic analysis. No sexual dimorphism had been previously reported in its AL (Schneiderman *et al.* 1982; Boeckh & Tolbert 1993; Galizia *et al.* 1999; Laissue *et al.* 1999). We found, in *D. melanogaster*, significant sexual

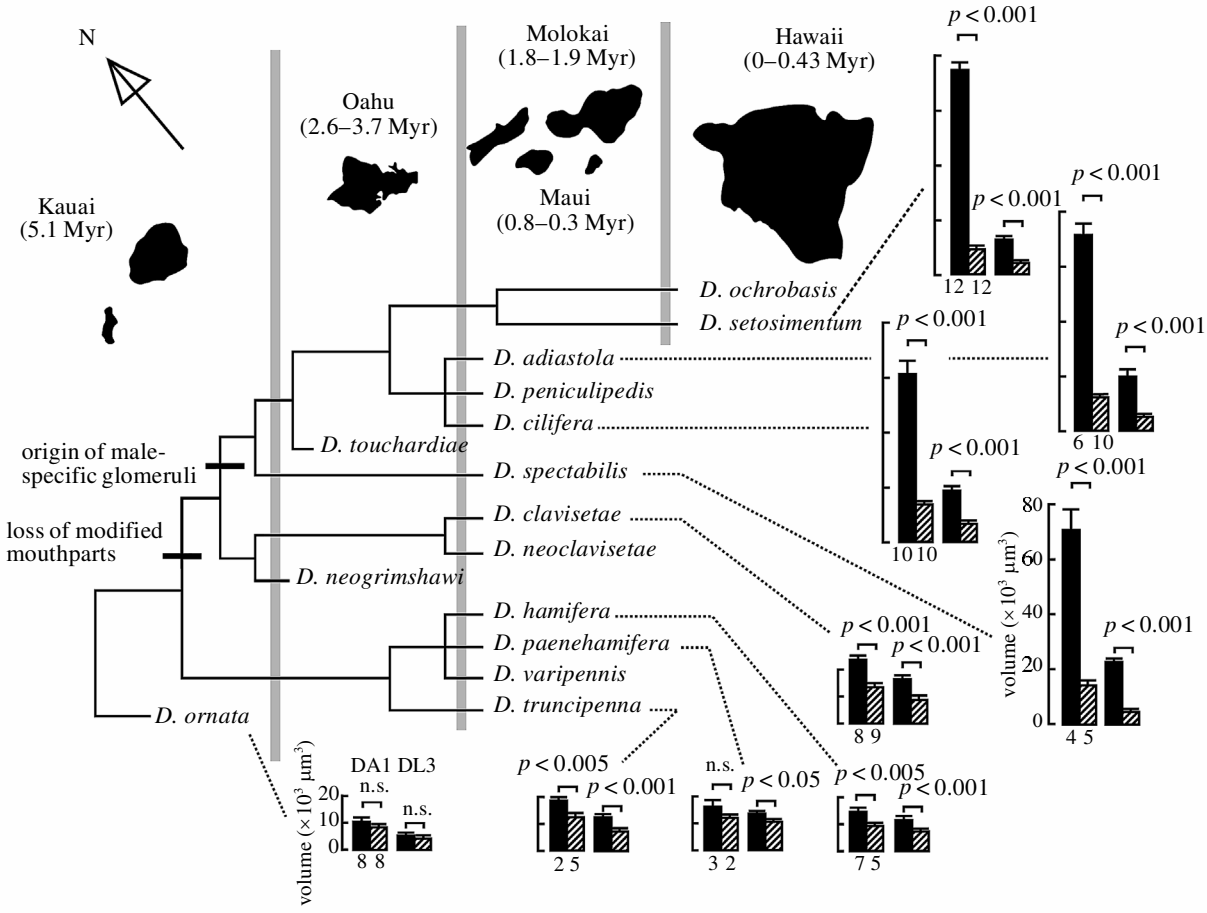

Figure 4. Evolutionary changes in the size of DA1 and DL3 among the *adiastola* subgroup species. Black and hatched bars represent the mean + s.e. volumes ($\times 10^3$ μm^3) of the glomeruli from male and female flies, respectively. The number of flies sampled is indicated by the number at the bottom of each bar. *D. ornata* from Kauai, the putative founder of the *adiastola* subgroup species, shows no significant size differences in DA1 ($p > 0.05$, two-tailed *t*-test) or DL3 ($p > 0.05$) between the two sexes.

dimorphism in two glomeruli, DA1 and VA1v, although the extent was less extreme than that found in the Hawaiian species (figures 2*a* and 5*a*). The mean volume of DA1 in one-week-old wild-type *D. melanogaster* (Canton-S) was 62% larger in male flies ($8.4 \pm 0.4 \times 10^3$ μm^3, $n = 20$) than in female flies ($5.2 \pm 0.2 \times 10^3$ μm^3, $n = 19$; $p < 0.001$), while that of VA1v was 33% larger in male flies ($6.7 \pm 0.2 \times 10^3$ μm^3) than in female flies ($5.0 \pm 0.2 \times 10^3$ μm^3; $p < 0.001$). No size differences between the sexes were found in two juxtaposed glomeruli, DL3 ($p = 0.31$) and VA1d ($p = 0.32$).

DA1 and VA1 are innervated by very early and late-born projection neurons, respectively (Jefferis *et al.* 2001). These two glomeruli were reported to be unique in that two different groups of projection neurons, as defined by the cell body locations, innervate these glomeruli (Marin *et al.* 2002; Wong *et al.* 2002). This contrasts with all other glomeruli examined, where only projection neurons belonging to a single group contributed to the innervation. The DA1 glomerulus is innervated by the lateral and ventral group neurons, while the VA1lm (in which our VA1v is included) glomerulus is innervated by the dorsal and ventral group neurons (Marin *et al.* 2002; Wong *et al.* 2002). It is intriguing to find that the projection neurons of the DA1 ventral group and those of the VA1lm ventral group are strikingly similar to each other in their axon patterns, differing from other projection neurons in the same glomeruli (Marin *et al.* 2002). It is tempting to speculate that these ventral group neurons are responsible for the observed sexual dimorphism in *D. melanogaster*.

In *D. melanogaster*, most sexual characteristics are formed under the control of the sex-determination cascade of genes (Marin & Baker 1998; Yamamoto *et al.* 1998; Ferveur & Greenspan 1998; Arthur *et al.* 1998; Yamamoto & Nakano 1999; Usui-Aoki *et al.* 2000). One of the key players in this cascade is *transformer* (*tra*). The female-type transcript of *transformer* (*tra*^{f}) encodes a protein with a feminizing effect, while the male-type transcript is unable to produce a functional protein. Thus, ectopic expression of *tra*^{f} in males results in a sexual transformation of the expressing cells. We expressed a UAS-driven *tra*^{f} cDNA construct (UAS-*tra*^{f}) in restricted body areas where Gal4 is expressed by means of *Gal4* enhanced trap insertions (Ferveur & Greenspan 1998). Enhancer trap lines used included *69B-Gal4*, *esg-Gal4*, *Dll-Gal4* and *53B-Gal4*. A striking effect of *tra*^{f} expression was observed when induced by *69B-Gal4* (figure 5*d*), but not when indicated by *esg-Gal4*, *Dll-Gal4* or *53B-Gal4*. The DA1 volume in male flies was dramatically reduced, to a size comparable with the female counterpart (the mean DA1 volume in males was $6.0 \pm 0.3 \times 10^3$ μm^3 ($n = 8$), while in females it was $6.2 \pm 0.2 \times 10^3$ μm^3 ($n = 9$); the difference

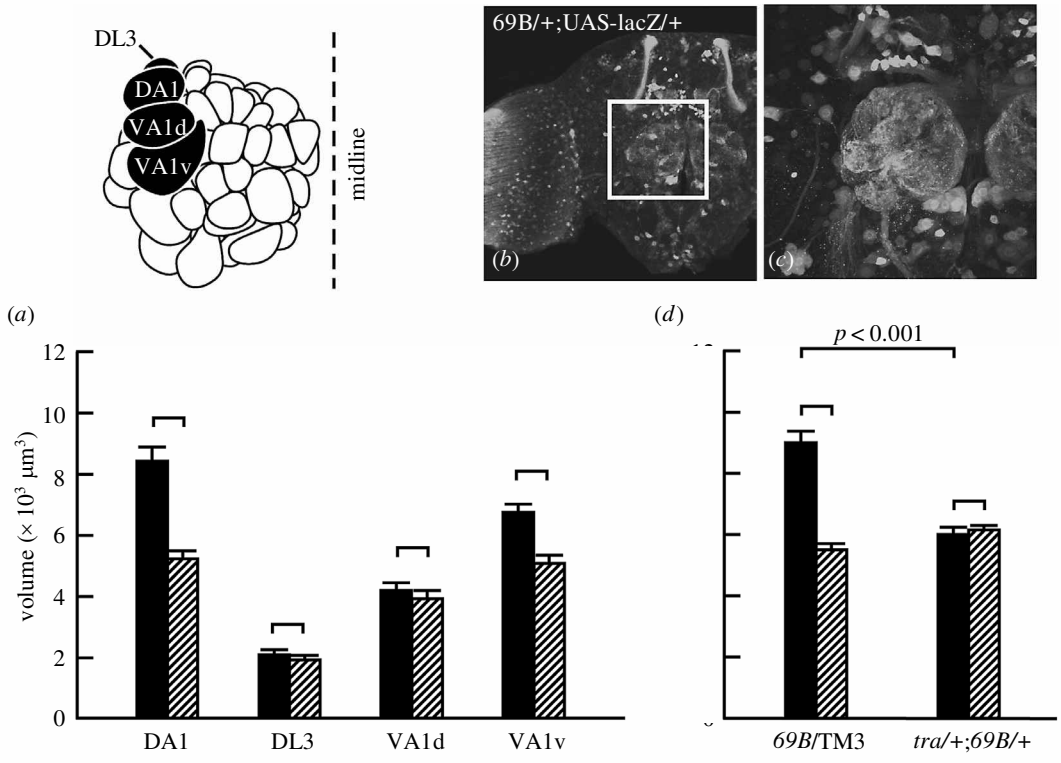

Figure 5. (*a*) The differences in the volumes of the DA1, DL3, VA1d and VA1v glomeruli, located in the anterior lateral region of the AL neuropil, in one-week-old male (black bars) and female (hatched bars) flies of wild-type *Drosophila* (Canton-S). Bars represent the mean + s.e. (× 10^3 μm³) volumes obtained from 20 males and 19 females. The DA1 and VA1v glomeruli are 62% and 33%, respectively, larger in males than in females whereas no sexual difference is observed in the sizes of the DL3 and VA1d glomeruli. (*b*,*c*) Confocal photomicrographs showing Gal4 expression patterns of *69B-Gal4* line in (*b*) the brain and (*c*) the AL of an adult fly, as revealed by the *UAS-lacZ* reporter. Frontal views of a brain whole mount. The *lacZ* expression was visualized by immunolabelling with an anti-β-galactosidase (β-gal) antibody and a Cy2-conjugated anti-rabbit antibody. (*d*) Targeted expression of tra^f in the Gal4 expressing domain of the *69B-Gal4* line of a UAS-*tra* strain. Bars represent the mean + s.e. (× 10^3 μm³) DA1 volume, averaged for six males and six females in the *69B-Gal4*/TM3 strain, and eight males and nine females in the *UAS-tra*/+ and *69B-Gal4*/+ strains, respectively. The ectopic expression of tra^f resulted in a remarkable reduction in DA1 volume, while no significant difference between male and female flies was observed in the strain *UAS-tra*/+; *69B-Gal4*/+. The difference in the DA1 volume between the control male and the *tra*-expressing male was statistically significant ($p < 0.001$).

was not significant at $p = 0.05$). In the *69B-Gal4* enhancer trap line, *Gal4* is expressed in many cells of epidermal lineage, including the antennal sensory neurons that project their axons into the AL glomeruli (figure 5*b*,*c*).

Some central neurons also express Gal4 in this enhancer trap line. Owing to the wide range of Gal4 expression in *69B-Gal4*, we were unable to specify the cells that mediated the tra^f activity to reduce the volume of the DA1 glomerulus in the male. We provided, however, unequivocal evidence that the sexual dimorphism in glomerular size in the AL is under the control of the sex-determination cascade, as are many other sex-specific characteristics of *Drosophila*.

The present study demonstrates that two identified glomeruli, in certain endemic Hawaiian *Drosophila*, have a much larger volume in males than in females. The systematic analysis of the glomerular organization across the fly phylogeny has allowed us to conclude that the male-specific enlargement of homologous glomeruli occurred independently in separate phylogenetic branches. This finding does not support the notion that the male-specific glomerulus is monophyletic. However, the formation of the male-specific glomerulus in phylogenetically distant species cannot merely be a result of functional convergence, because the glomeruli that show a male-specific enlargement in different species are anatomically homologous.

Interestingly, the DA1 glomerulus in the *Drosophila* AL is located anterolaterally near the base of the antennal nerve, a position similar to that of the macroglomerular complex in moths (Schneiderman *et al.* 1982) and cockroaches (Boeckh & Tolbert 1993). Although the idea that the *Drosophila* DA1 glomerulus is anatomically homologous to an element of the macroglomerular complex in larger insects is attractive, no evidence is available, thus far, to support or reject this possibility.

Among endemic Hawaiian *Drosophila*, the most conspicuous sexual dimorphism in the AL was found in the *adiastola* subgroup of the picture-wing species. Mature males of the picture-wing species advertise their sexual readiness by characteristic displays on their leks. Within the lek, males of the *adiastola* subgroup exhibit a characteristic posture during a sequence of courtship displays: proboscis extension toward the female's face with the labellar lobes fully opened, wing extension, curling the abdomen upward and over the thorax and, often, pulsation of an anal droplet during the display (Spieth 1987). It may be postulated that the males of these species require a higher olfactory acuity than females to be successful in

such elaborate mating behaviour (Spieth 1987; Tompkins *et al.* 1993). If the DA1 glomerulus is involved in female pheromone processing, as is the case for the macroglomerular complex in larger insects, then sexual selection may favour males with an enlarged glomerulus for improved olfactory performance. The contribution of olfaction to mating behaviour in these species could be assessed by, for example, observations of flies in which the antennal sense organs had been surgically ablated.

A recent imaging study has revealed the olfactory responses of individual glomeruli when stimulated with an apple, cherry or banana odour: DA1 does not respond to any of the stimuli; VA1 is activated by apple and banana; and DM3 is responsive to all three odours (Ng *et al.* 2002). It may be that DA1 is involved in the detection not of food, but of odours relevant to other aspects of the fly's life, including sexual behaviour.

In this study, we document that the DA1 glomerulus is also sexually dimorphic in *D. melanogaster*. This finding establishes a model organism for the genetic dissection of brain sexual dimorphism. As a first step, we demonstrated that the enlargement of DA1 is prevented by the action of female-determinant Tra in males. This result suggests that sexual dimorphism in the glomerular volume is tightly regulated by the sex-determination network of genes. The feminization of the DA1 glomerulus was probably a result of tra^{f} expression in primary sensory neurons, although tra^{f} effects via central neurons cannot be excluded. It is of interest to note here that an increase in the number of sensory-neuron synapses induced by the gigas mutation increased the volume of the postsynaptic glomeruli in the AL of *D. melanogaster* (Acebes & Ferrus 2001). This increase in the glomerular volume was accompanied by an elevated olfactory sensitivity to a given odour, as determined by a behavioural assay (Acebes & Ferrus 2001).

An experiment to feminize the male glomeruli has been reported in the moth *Manduca sexta* using an anatomical method. A female AL that is innervated by olfactory-cell axons from a grafted male antenna may develop a macroglomerular complex with three glomeruli, similar to that of a normal male AL (Schneiderman *et al.* 1982, 1986; Rossler *et al.* 1999). Together, these results raise the intriguing possibility that a defined set of glomeruli (e.g. DA1, DL3 and VA1v in *Drosophila*, or components of the macroglomerular complex in larger insects) can enlarge if they are innervated by sex-specific (male specific in this case) sensory afferents, whose differentiation is regulated by the sex-determination genes.

Although the sexual dimorphism in the volume of the AL glomeruli has a genetic basis, it may also be affected by some epigenetic factors. Indeed, the exposure of flies to certain odours in early adult life is known to induce a long-lasting reduction in the volume of specific glomeruli (Devaud *et al.* 2001). Such experience-dependent modification might have hampered the detection of sexual dimorphism in the glomerular volume in preceding studies with *D. melanogaster*.

Recently, it was shown that sexually dimorphic expression of the *bric-a-brac* (*bab*) gene directly leads to male-specific pigmentation of abdominal segments A5 and A6 in all members of the *melanogaster* subgroup but not in some species of the *montium* subgroup, although the latter species also show sexual dimorphism in *bab* expression (Kopp *et al.* 2000). It is thus possible that the sex-determination signal establishes sexually dimorphic gene expression in given glomeruli and associated sensory neurons, without inducing their enlargement in males. The hypothesis of 'genetic competence' to sex-specific enlargement of certain glomeruli can now be tested by manipulating sensory-neuron differentiation using the large collection of developmental mutations available in *D. melanogaster*.

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