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Genetics

The muscle of Lawrence in *Drosophila*: A case of repeated evolutionary loss

(phylogeny)

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ABSTRACT The muscle of Lawrence (MOL) is a bilaterally symmetrical muscle spanning the tergite of the fifth abdominal segment of adult male *Drosophila melanogaster*. It is not, however, a general feature of male-specific development within the subfamily Drosophilinae. Of 95 species surveyed within this subfamily, 67 exist with no MOL at all. By drawing comparisons with published cladograms of species relatedness, three conclusions regarding the evolutionary history of the MOL are made: (*i*) The MOL predates the major radiations of the genus *Drosophila,* given its presence in earlier-branching *Chymomyza* and *Scaptodrosophila*; the MOL has been subsequently excluded in at least one present species of each of these two primitive genera. (*ii*) Within the genus *Drosophila* the MOL is present sporadically in the radiation of the subgenus *Sophophora*, showing repetitive loss even in very close evolutionary lineages. (*iii*) The MOL may have been entirely excluded from the prolific radiation of the subgenus *Drosophila*. Thus the MOL shows a uniquely incongruous pattern of presence or absence relative to accepted drosophilid phylogeny.

First described in *Drosophila melanogaster,* the muscle of Lawrence (MOL) is a large, bilaterally symmetrical muscle spanning the fifth tergite of the abdomen of adult males; it does not develop in females (1). Oddly, the MOL eluded decades of extensive genetic and biological investigation in *D. melanogaster*, including the definitive survey of adult musculature by Miller in 1950 (2). Only in 1984 was this muscle's existence first revealed with the report of Lawrence (the muscle's eponym) and Johnston (1), showing that formation of the MOL is sex-specific and is associated with developmental cues in the fifth abdominal segment (A5) of the male; homeotic mutations transforming either A4 or A6 to A5 induce the development of a MOL in the transformed segment (1, 3).

Development of the MOL depends not on the sex of its progenitor myoblasts, but rather the sex of the contacting motoneuron in A5. If the motoneuron is male, the MOL develops; if the motoneuron is female, the MOL fails to develop (3). Ablation of the ingrowing motoneuron in a male correspondingly blocks MOL development (4). Although use of the MOL to the adult male fly is unknown, other interesting observations have accumulated regarding its general biology, including genetic evidence that its development requires normal expression of *fruitless (fru)* (5, 6), a gene identified by its involvement in adult male courtship behavior (7) and as a sex determination gene that acts late in development (8, 9). In addition, formation of the MOL in A5 involves recruitment of the relatively rare 79B actin (10).

To gain insight into the evolutionary history of this muscle, we have analyzed its appearance within the subfamily Drosophilinae. Surprisingly, of 95 species surveyed, 67 have no MOL at all. By comparing our observations with generally accepted cladograms of species relatedness within the Drosophilinae (11–24), we provide evidence that the MOL was an existing feature of primitive forms within the early radiation of the subfamily. Also stemming from that comparison is our conclusion that several independent genetic exclusions of this muscle have occurred during the subsequent radiation of MOL-containing lines. This makes the MOL a rare example of an anatomical structure that has undergone independent, repeated loss among closely related evolutionary lines.

MATERIALS AND METHODS

All fly stocks were maintained on an instant cornmeal-based medium. Species other than *D. melanogaster* were obtained from the National *Drosophila* Species Resource Center, Bowling Green, OH.

The techniques for preparation of specimens and visualization of their musculature by birefringence under polarized light have been previously described (5). For each of the eight species of the *melanogaster* species subgroup, 20 males and 5 females were dissected and analyzed. For species outside the *melanogaster* species subgroup, 10 males and 5 females were dissected in each species, and then their musculature compared. For a species to be scored as MOL-absent, there was no discernible difference in the male and female A5 musculature. No MOL or MOL-like structure was ever observed in a female (n = 475 females dissected).

One set of experiments involved hybridization of males from a MOL-containing species to females of species whose males do not develop the MOL. Such crosses generally performed poorly and were optimized by crowding parents. For each hybrid cross, 10 males were assessed for their abdominal musculature. Their hybrid male genotypes were ascertained first by sterility with virgin females of both parental species (25) and then by verification of hybrid anatomy of the genital arch.

RESULTS AND DISCUSSION

The MOL Is Not a General Anatomical Feature of All *Drosophila* Males. The MOL is shown in Fig. 1*a* as it normally appears in the dorsal abdomen of adult male *D. melanogaster*. Could this muscle be a general requirement for normal mating

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Abbreviations: MOL, muscle of Lawrence; A5, fifth abdominal segment; *fru*, *fruitless*.

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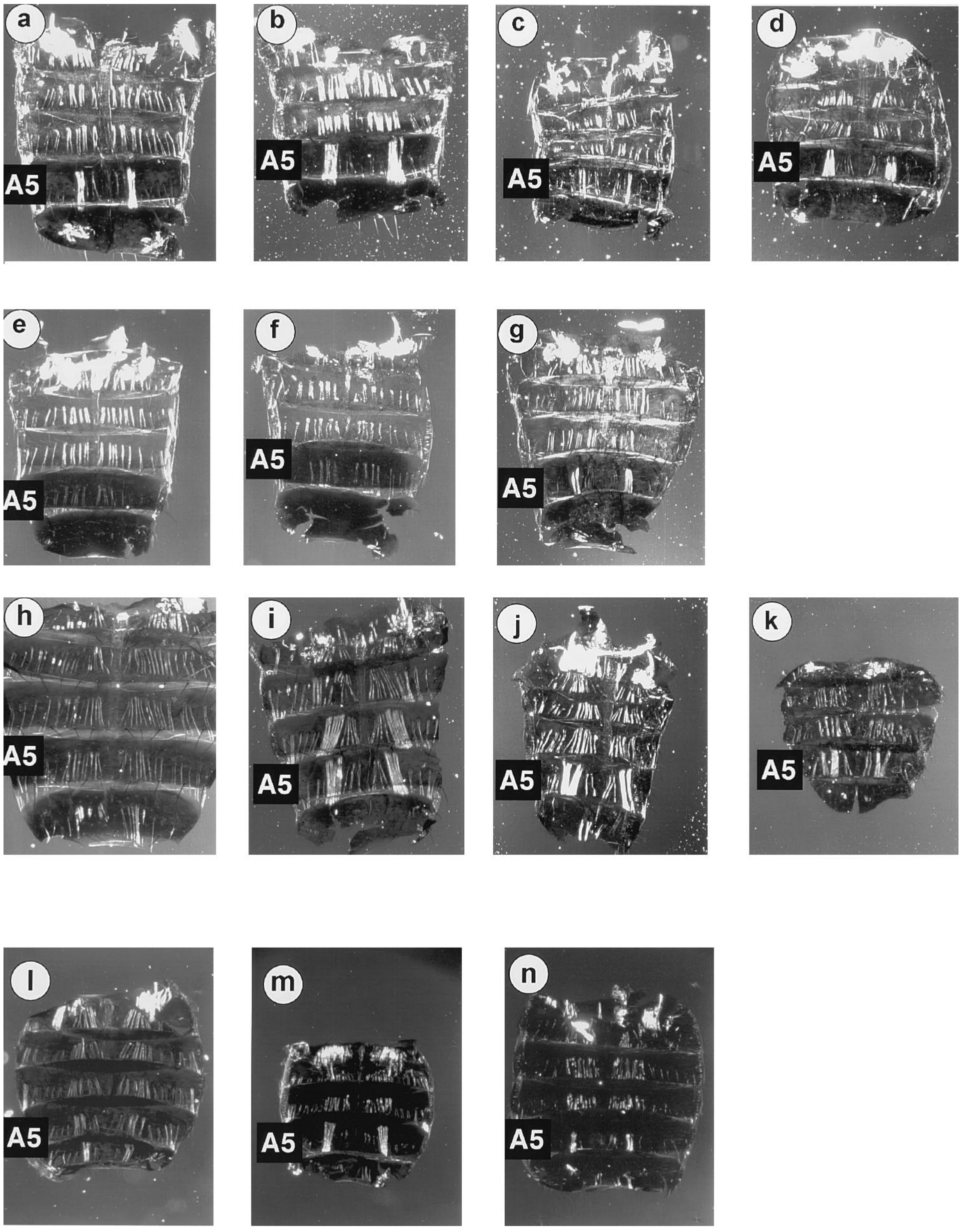

FIG. 1. Comparative dorsal abdominal musculature among adult drosophilid males and females. (*a*) *D. melanogaster* male. (*b*) *D. mauritiana* male. (*c*) *D. simulans* male. (*d*) *D. sechellia* male. (*e*) *D. yakuba* male. (*f*) *D. erecta* male. (*g*) Hybrid male from the cross *D. mauritiana* males × *D. teissieri* females (hybrid males from the cross *D. mauritiana* males × *D. yakuba* females also contained a MOL, not shown). (*h*) *D. subobscura* female. (*i*) *D. subobscura* male. (*j*) *D. pseudoobscura* male. (*k*) *D. azteca* male. (*l*) *Chymomyza amoena* female. (*m*) *Chymomyza amoena* male. (*n*) *Scaptodrosophila lebanonensis* male. When present, note especially the species-specific variation of the MOL in terms of fiber size and number. Consistent among all species with the MOL are its obvious length, size, position relative to the smaller, adjacent longitudinal fibers, and insertion in A6 (5), apart from the *D. subobscura* male, which shows a duplication of the MOL in A4 (*i*).

ability and fertility of all males within the genus? This question was first addressed only with great difficulty by generating two *fru* mutant *D. melanogaster* males who developed no MOL, yet were fertile (5). Here, the answer is a straightforward no. Almost three-fourths of the *Drosophila* species surveyed in this study showed no evidence of MOL development (61 of 84 species; Table 1, Fig. 2). Noting that there are more than 1,600 reported species of *Drosophila* (22), most of which are not easily available for analysis, our survey could not be exhaustive. Nonetheless, our results point first to an apparent division in MOL presence among the four largest species groups of the subgenus *Sophophora*. The MOL seems to appear exclusively within the *obscura* and *melanogaster* groups, whereas no species within the *saltans* and *willistoni* groups was found with a MOL (Fig. 2). The following points are particularly noteworthy.

Not every species of the *obscura* and *melanogaster* groups that was examined had a MOL. Whereas 8 of 9 species dissected from the *obscura* species group contained a MOL, only 15 of 41 from the *melanogaster* species group did (Fig. 2).

Conspicuously within the *melanogaster* group, dissection of half the species of the *ananassae* species subgroup, and about one-sixth of the many species of the *montium* species subgroup, did not reveal a species with the MOL (Fig. 2). Likewise, analysis of nearly half the species of the *saltans* and nearly all the species of the *willistoni* species groups revealed no MOL (Fig. 2). Consequently, these may represent sophophoran lines in which the MOL is completely missing.

Also conspicuous was our finding that this unusual pattern of MOL presence and absence carried through even to the *melanogaster* species subgroup, in which 4 of the 8 species had the MOL, but 4 did not (Fig. 1 *a–d* vs. *e* and *f*; Fig. 2).

Table 1. Listing of species analyzed for MOL phenotype

Genus	Subgenus	**Species group**: *Species*
Chymomyza (MOL^{+}): *amoena*		
Chymomyza (MOL^{-}): *procnemis*		
Drosophila		
	Dorsilopha (MOL^{-}): *busckii*	
	Drosophila (MOL^{-})	
		annulimana: *aracatacas*; ***cardini***: *cardini*; ***funebris***: *funebris*; ***immigrans***: *immigrans*, *nasuta*; ***melanica***: *melanica*; ***mesophragmatica***: *gaucha*; ***modified mouth parts***: *mimica*; ***nannoptera***: *nannoptera*; ***quinaria***: *palustris*; ***repleta***: *arizonensis*, *mercatorum mercatorum*, *mulleri*; ***robusta***: *robusta*; ***tripunctata***: *tripunctata*; ***virilis***: *virilis*
	Sophophora	
		melanogaster (MOL^{+}): *elegans*, *eugracilis*, *lucipennis*, *lutescens*, *mauritiana*, *melanogaster*, *mimetica*, *paralutea*, *prostipennis*, *pseudotakahashii*, *pulchrella*, *rajasekari*, *sechellia*, *simulans*, *takahashii*
		melanogaster (MOL^{-}): *ananassae*, *auraria*, *baimaii*, *bipectinata*, *ercepeae*, *erecta*, *ficusphila*, *jambulina*, *kikkawai*, *lacteicornis*, *lini*, *malerkotliana*, *mayri*, *orena*, *pallidosa*, *parabipectinata*, *pennae*, *phaeopleura*, *pseudoananassae*, *punjabiensis*, *quadraria*, *rufa*, *seguyi*, *teissieri*, *varians*, *yakuba*
		obscura (MOL^{+}): *ambigua*, *azteca*, *bifasciata*, *miranda*, *persimilis*, *pseudoobscura*, *subobscura*, *tolteca*
		obscura (MOL^{-}): *affinis*
		saltans (MOL^{-}): *austrosaltans*, *emarginata*, *lusaltans*, *milleri*, *neocordata*, *prosaltans*, *saltans*, *sturtevanti*, *subsaltans*
		willistoni (MOL^{-}): *capricorni*, *equinoxialis*, *fumipennis*, *nebulosa*, *paulistorum*, *succinea*, *tropicalis*, *willistoni*
	Hawaiian "*Drosophila*" (*Idiomyia*) (MOL^{-}): *crucigera*, *gymnobasis*	
	Hirtodrosophila (MOL^{-}): *pictiventris*	
	Scaptodrosophila (MOL^{+}): *dimorpha*, *lebanonensis lebanonensis*, *pattersoni*, *stonei*	
	Scaptodrosophila (MOL^{-}): *latifasciaeformis*	
	Zaprionus (MOL^{-}): *tuberculatus*	

Genera designations (23); subgenus *Drosophila* species groups (21); see legend, Fig. 2 for other groupings.

Remarkably, however, hybrid males can be generated in certain crosses between one of these MOL containing species and a species with no MOL (Fig. 1*g*; also see below).

When present, the *melanogaster* species group MOL was homogeneous in appearance and within the narrow range of forms as displayed by the *melanogaster* species subgroup (Fig. 1 *a–d*). By comparison, the *obscura* species group MOL varied widely in appearance. The most striking case was *D. subobscura* in which the MOL is duplicated into A4 (Fig. 1 *h* vs. *i*). The MOL of *D. pseudoobscura* is more like that of *D. melanogaster* (Fig. 1 *j* vs. *a*), whereas the MOL of *D. azteca* is more weakly formed (Fig. 1*k*).

MOL Presence, Then Absence: A Case of Repeated Evolutionary Loss. *Phylogeny and the MOL within the subfamily Drosophilinae.* Whether assessing criteria as diverse as morphological characteristics or DNA sequence, only a few drosophilid groups raise controversy regarding their phylogenetic placement (cf. 20, 22–24). Fig. 3 shows a current drosophilid phylogeny, based on extensive morphological comparisons (23), and includes the corresponding MOL analysis.

The *Chymomyza* and *Scaptodrosophila* genera represent two of the earliest radiations within the subfamily Drosophilinae (23); present-day species of both show the MOL (Fig. 3, Table 1). The early split of these two genera has been substantiated by molecular comparison of introns and coding sequences of the superoxide dismutase (*Sod*) gene (24). Thus we conclude that the MOL, as an anatomical structure, predated all the major *Drosophila* radiations. By their phylogeny, both *Chymo-*

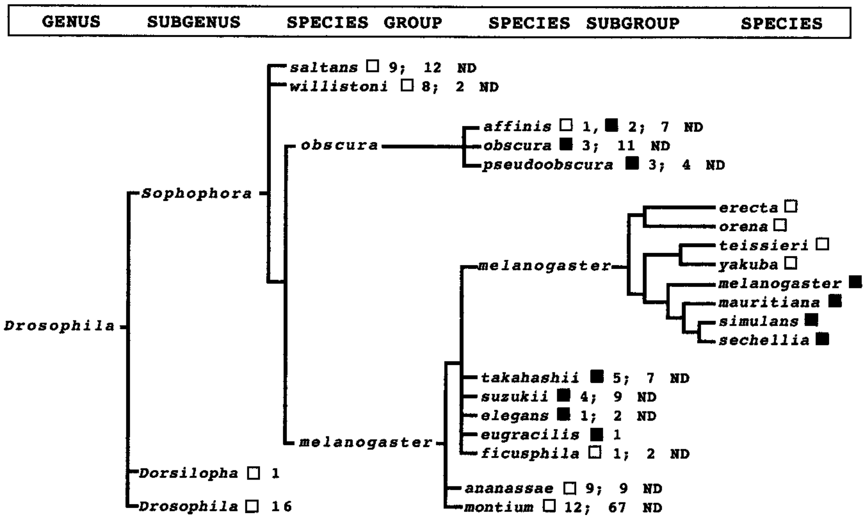

FIG. 2. Distribution of the MOL in *Drosophila* species analyzed in this study. Note that a complete cladogram is shown for the *melanogaster* species subgroup only. This is a consensus cladogram based on allozyme, chromosomal and behavioral comparisons (12); a very similar phylogeny based on DNA hybridization criteria also has been reported (13). See references below for specific details regarding other groupings. ☐, MOL absent; ■, MOL present; ND, not dissected. To interpret particular results, the *saltans* species group is explained as example: Of 21 total species within the group, 9 of 9 dissected contained no MOL; 12 species were not dissected. For species outside the *melanogaster* species subgroup $n = 10$ males and 5 females dissected per species; within the *melanogaster* species subgroup $n = 20$ males and 5 females per species. Groupings are based on the following general literature: *melanogaster* subgroup (11–13); *melanogaster* group (14); *saltans* group (15); *willistoni* group (16); *obscura* group (17–19); *obscura* and *melanogaster* group cluster (20, 21); general reviews on evolution within the family Drosophilidae (20–24).

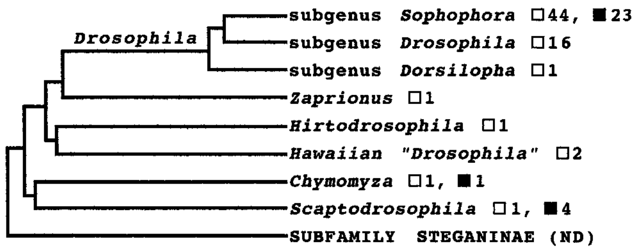

FIG. 3. Phylogenetic relationship of the major genera within the subfamily Drosophilinae and MOL analysis (23). *Hirtodrosophila* and *Zaprionus* have been alternatively placed within the *Drosophila* genus radiation (24). □, MOL absent, ■, MOL present in a particular group. A single lineage with both symbols contains species with and without the MOL.

myza and *Scaptodrosophila* conspicuously show evidence of independent loss of the MOL (Fig. 3, Table 1). There is also a distinct phenotypic variability of the MOL within these two genera. The *Chymomyza* MOL appears more like that of the *melanogaster* species subgroup (compare first the *Chymomyza* female vs. male, Fig. 1 *l* and *m*; then Fig. 1 *m* vs. *a*–*d*). By comparison, the MOL fibers of *Scaptodrosophila* species are consistently shorter, approximately the length of adjoining longitudinal fibers (Fig. 1*n* is representative of the four *Scaptodrosophila* species that were found with a MOL; Table 1).

Subsequent to the *Chymomyza-Scaptodrosophila* radiations, but still before those of the genus *Drosophila*, are branches leading to the genera Hawaiian "*Drosophila*" (or *Idiomyia*), *Hirtodrosophila* and *Zaprionus*, as well as the subgenus *Dorsilopha* (23). Our anatomical analysis of these four taxa revealed no MOL (Fig. 3, Table 1). Such a pattern again necessitates independent MOL losses, at the least, at three more evolutionary branch points (Fig. 3).

Hirtodrosophila and *Zaprionus* have been alternatively placed, based on their *Sod* gene structure, within the radiation leading to the subgenus *Drosophila* (24). This might reveal the constancy of a single evolutionary lineage in which the MOL has been entirely excluded, since again, no species of any of these three lines was found with a MOL (Figs. 2 and 3). This includes 16 species of the subgenus *Drosophila*, representing 13 species groups (21). But whether these entire taxa are MOL-less is speculative, because our conclusion is based on these few species (Table 1, Fig. 3). For example, the subgenus *Drosophila* radiation has been by far the most prolific, evolving more than 800 species (22).

Could the MOL be a general feature of dipteran development? It might be possible ultimately to reveal the MOL's evolutionary origin by analyzing ever-earlier radiations. This should include species of the drosophilid subfamily Steganinae (20, 23), before moving outside Drosophilidae into other families of the order Diptera. We have broached this question with dissection of one tephritid (*Rhagoletis completa*) and one muscid (*Musca domestica*); both had no MOL.

Phylogeny and the MOL within the subgenus Sophophora. The simplest evolutionary model would predict that with the appearance of an anatomical structure such as the MOL it might be transmitted in a linear fashion to all species radiating from that point forward in time. Thus, if the MOL were present as early as the *Chymomyza* and *Scaptodrosophila* radiations, one ought to uncover a direct lineage in which all radiating species have the structure. This is certainly the case with male-specific sex combs. This is an anatomical feature found in all species of the *obscura* and *melanogaster* species groups, but in no other *Drosophila* (21). Regarding the MOL, this evolutionary scenario does not hold, from the primitive genera of the subfamily, to the *melanogaster* species subgroup (Figs. 2 and 3). Consequently we conclude that the sporadic and frequent absence of the MOL as we have recorded in the radiation of the entire subfamily Drosophilinae is due to evolutionary loss of the muscle not once, but repeatedly. This becomes especially evident within the subgenus *Sophophora.*

Perhaps the most striking loss is seen within the close lineage of the eight species of the *melanogaster* species subgroup. The cladogram displayed in Fig. 2 for this subgroup is a composite based on allozyme analysis at many enzyme loci, with complementing chromosomal and behavioral comparisons (11, 12), and if correct, necessitates two independent evolutionary losses of the MOL (at the branch point of the *erecta* cluster and at the branch point of the *yakuba* cluster; Fig. 2). Note that a very similar cladogram of the subgroup has been derived from DNA hybridization studies (13); this grouping also requires two independent exclusions of the MOL.

The only constancy of lineage appears within four species subgroups considered close to the *melanogaster* species subgroup: *takahashii*, *suzukii*, *elegans* and *eugracilis* (12). Of the 29 total species in this cluster, the 11 we have surveyed all contain a similar-appearing MOL. But if the grouping here of the *ficusphila* species subgroup is correct (12), then another incidence of evolutionary loss must be invoked (Fig. 2).

A similar case arises within the *obscura* species group (Fig. 2). With about one-fourth of the reported species in this group analyzed (17–19), only one was found to be lacking the MOL; this must have involved yet another independent case of MOL loss. As noted, the appearance of the MOL in this group is much more variable than in the *melanogaster* group (Fig. 1). This could correlate with the early branching of these two groups during the sophophoran radiation (24). For whatever reason, the form of the *obscura* MOL is not fixed and conserved, and this variability should provide important insight as additional features of the MOL, its physiology, and evolutionary history are investigated.

Our analysis of the *melanogaster* species-group branch-point, leading to the *ananassae* and *montium* species subgroups, indicates at least one more point of MOL loss (Fig. 2). Taken together, these instances of independent MOL loss within the *Sophophora* radiation correlate with the MOL losses we have documented in *Chymomyza* and *Scaptodrosophila.*

Alternative hypotheses regarding the evolution of this muscle are less tenable: that the MOL has arisen independently more than once, or that published cladograms—based on extensive morphological, chromosomal, behavioral, allozyme, and DNA sequence analysis (11–24)—are fraught with errors.

When present in a given species, the MOL phenotype was fully penetrant and generally did not vary among males. One exception was *D. miranda*, a member of the *obscura* species group (Table 1). Nine of 10 males had a similar MOL in A5, whereas one male had only a unilateral MOL. Another exception was *Scaptodrosophila lebanonensis*. In this species nine of 10 males showed clustering and/or thickening of fibers in A5 (as in the male pictured in Fig. 1*n*), whereas one male showed a complete absence of the MOL. Given their general rarity, these could be indications of species segregating more than one MOL phenotype in males.

Hybrid Analysis Within the *melanogaster* Species Subgroup. Given that four species of the *melanogaster* species subgroup have the MOL while four do not (Fig. 2), it could be the case that the change from the MOL-present to the MOL-absent state could have a simple genetic basis. We tested this indirectly by generating hybrid males (25) from *D. mauritiana* fathers (a MOL-containing species), crossed to either *D. yakuba* or *D. teissieri* mothers (species containing no MOL). All 10 hybrid males analyzed from each cross contained a MOL (Fig. 1*g*; only the *D. mauritiana* × *D. teissieri* hybrid male is shown). These crosses provide two important pieces of information: (*i*) the *D. mauritiana* autosomes are sufficient for triggering the development of the MOL when in combination with either MOL-less genome; and (*ii*) it is unlikely that either *D. yakuba* or *D. teissieri* lack the MOL due to the evolution of a dominant suppressor of MOL development (assuming that such a suppressor would be capable of function in the *D. mauritiana* genetic background). It could be that a MOL-less genetic background is recessive to the genetic cues triggering MOL development that reside on MOL-containing species' autosomes. This implies that within this subgroup the genetic differences between the MOL-present and the MOL-absent states are indeed minimal.

***fru* Effects on the MOL.** Perhaps MOL variation is influenced by evolutionary divergence of the informational content or expression pattern of *fru*. The interrelationship between MOL development and the *fru* locus is genetically defined by four *fru* alleles, which in varying degrees result in aberrations of at least two aspects of male courtship behavior (7). The fru^1 and fru^2 mutations affect the development of the MOL, leading to an incompletely developed structure in most individual males (5). The fru^3 and fru^4 mutations, tagged transposon insertions in the *fru* locus that lead to mutant *fru* behavior (7, 26), also completely block expression of the MOL when either is homozygous, or when in heterozygous combination (A.V., D.A.G., B. Berwald, S.O., P. T. Barnes, and J.C.H., unpublished results). The etiologies of these mutant effects are likely to be vastly different at the molecular level relative to the establishment of behavioral patterns vs. regulation of the development of a muscle. This is consistent with the notion that *fru* itself is a master male-behavior and MOL-determinant gene, and that *fru* expression is controlled in part by the *transformer* gene of the sex determination cascade (9).

Evolutionary addition or loss of the MOL may have directly involved the *fru* locus. With the recent cloning and molecular characterization of *fru* (6, 9), the evolutionary question just implied can be addressed experimentally. In this respect our hybrid analysis is tantalizing. Given the recent evolution of the *melanogaster* subgroup species it is unlikely that the change from the MOL-present to the MOL-absent state, or vice versa, involved many genetic alterations. Thus molecular comparisons of *fru* among *Drosophila* species might reveal a correlation.

CONCLUSIONS

The MOL is a dorsal abdominal muscle occurring in the males of a limited range of drosophilid species. It shows an unusual pattern of presence and absence that we have interpreted as repeated evolutionary loss. What makes the MOL a uniquely evolving structure, however, is its apparent loss even within closely related evolutionary lineages. What results is a pattern of presence that does not match accepted drosophilid phylogeny.

The utility of this muscle to the male flies possessing it has yet to be elucidated. An answer is conceivable through comparative physiological and behavioral analysis. But so far, no male behavioral difference has been noted that correlates with presence or absence of the MOL, especially within the *melanogaster* species subgroup (27).

Nevertheless, the fact that it is possible genetically to uncouple male-specific MOL development from that of the remaining adult musculature may allow a unique opportunity to study the developmental processes underlying MOL formation, by an approach that combines evolutionary perspectives with molecular genetics.

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