

SHORT COMMUNICATION

Phylogenetic Relationships among Species Groups of the *virilis-repleta* Radiation of *Drosophila*

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One of the largest radiations within the Drosophilinae is the *virilis-repleta* radiation, as evaluated by the number of species and species groups (Throckmorton, 1962, 1975). The *virilis* and *repleta* species groups have been extensively used as model systems in the studies of mechanisms of speciation, mapping genes, and chromosomal rearrangements (Patterson and Stone, 1952; Powell, 1997). *Drosophila virilis* is a prominent reference species for comparison with *D. melanogaster* in regard to patterns and mechanisms of molecular and genomic evolution (Nurminsky *et al.*, 1996; Hartl and Lozovskaya, 1995). The *Drosophila repleta* group within this radiation is potentially very promising for the study of speciation mechanisms, because it displays a range of different degrees of reproductive isolation among its members (Patterson and Stone, 1952). It also provides an example of a very rapid and successful radiation in the deserts.

The phylogenetic relationships of species groups in the *virilis-repleta* radiation are largely unresolved in Throckmorton's classifications. Moreover, there are some discrepancies between his earlier (1962) and later (1975) schemes. Improvement in the understanding of the phylogenetic relationships among the species groups and subgroups of the *virilis-repleta* radiation has come from cytological studies (reviewed in Wasserman, 1982, 1992; Powell, 1997), but there were few molecular studies considering intergroup and subgroup relationships of the radiation. In a study based on 28S rRNA genes, Pélandakis and Solignac (1993) included representatives of many groups of the *virilis-repleta* radiation. With the exception of *D. polychaeta*, these groups form a monophyletic lineage, but with low bootstrap support (less than 50%) for the clade as a whole and for the relationships among species groups. Recently, Durando *et al.* (2000) have conducted a detailed phylogenetic analysis of the *repleta* species group, which also includes three other groups of the

repleta radiation. This analysis is informative about relationships within species complexes, but leaves relationships among the species complexes, subgroups, and groups largely unresolved.

We explore phylogenetic relationships of the *virilis-repleta* radiation using two nuclear genes, *Ddc* and *amd*, in representatives of 12 species groups and five subgroups of the *repleta* group. We address two main questions: (1) monophyly of the *virilis-repleta* radiation, and (2) phylogenetic relationships of species groups and subgroups to one another within the outlined clade.

A sample of 20 species that belong to the *virilis-repleta* radiation (*sensu* Throckmorton, 1975) are listed in Table 1. The strains of all species were provided by the National *Drosophila* Species Resource Center (Yoon, 1996). The *amd* and *Ddc* sequences are newly determined for 17 species (i.e., with the exception of *D. virilis*, *D. robusta*, and *D. hydei*, which are reported in Tatarenkov *et al.*, 2001). In order to verify the monophyly of the *virilis-repleta* radiation, we have used the *amd* and *Ddc* sequences of 18 additional species representing major Drosophilinae lineages. Of these additional species, the *Ddc* sequence in *D. tripunctata* is newly determined (GenBank accession number AF324964) and the *amd* sequence (AF293728) is from Tatarenkov *et al.* (2001). The *amd* (X04695) and *Ddc* (X04661) of *D. melanogaster* are, respectively, from Marsh *et al.* (1986) and Eveleth *et al.* (1986). The other sequences have been reported by Tatarenkov *et al.* (1999a,b, 2001). GenBank accession numbers for *amd* and *Ddc* are, respectively: *Scaptomyza adusta* (AF293704, AF293732), *S. palmae* (AF293718, AF293743), *D. gymnobasis* (AF293710, AF293735), *D. mimica* (AF293716, AF293741), *D. funebris* (AF293709, AF293734), *D. phalerata* (AF293721, AF293745), *D. immigrans* (AF293713, AF293738), *Samoaia leonensis* (AF293725, AF293748), *Zaprionus tuberculatus* (AF293731, AF293751), *D. (Hirtodrosophila) pictiventris* (AF293711, AF293736), *D.*

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TABLE 1

The 20 Species of the *virilis-repleta* Radiation (*sensu* Throckmorton, 1975) in This Study

Genus	Group	Subgroup	Species	Genbank accession no.	
				<i>amd</i>	<i>Ddc</i>
<i>Drosophila</i>	<i>repleta</i>	<i>hydei</i>	<i>hydei</i>	AF293712	AF293737
			<i>eohydei</i>	<u>AF324954</u>	<u>AF324970</u>
		<i>mulleri</i>	<i>mulleri</i>	<u>AF324958</u>	<u>AF324974</u>
			<i>buzzati</i>	<u>AF324947</u>	<u>AF324980</u>
		<i>melanopalpa</i>	<i>repleta</i>	<u>AF324961</u>	<u>AF324977</u>
			<i>canapalpa</i>	<u>AF324948</u>	<u>AF324981</u>
		<i>mercatorum</i>	<i>mercatorum</i>	<u>AF324957</u>	<u>AF324973</u>
			<i>fasciola</i>	<u>AF324953</u>	<u>AF324969</u>
		<i>mesophragmatica</i>	<i>gaucha</i>	<u>AF324955</u>	<u>AF324971</u>
			<i>camargoi</i>	<u>AF324951</u>	<u>AF324967</u>
		<i>dreyfusi</i>	<i>canalinae</i>	<u>AF324952</u>	<u>AF324968</u>
		<i>canalinae</i>	<i>bromeliae</i>	<u>AF324950</u>	<u>AF324966</u>
		<i>bromeliae</i>	<i>nannoptera</i>	<u>AF324959</u>	<u>AF324975</u>
		<i>nannoptera</i>	<i>aracatacas</i>	<u>AF324949</u>	<u>AF324965</u>
		<i>annulimana</i>	<i>virilis</i>	AF293729	AF293749
		<i>virilis</i>	<i>robusta</i>	AF293724	AF293747
		<i>robusta</i>	<i>sordidula</i>	<u>AF324963</u>	<u>AF324979</u>
		<i>melanica</i>	<i>melanica</i>	<u>AF324956</u>	<u>AF324972</u>
		<i>polychaeta</i>	<i>polychaeta</i>	<u>AF324960</u>	<u>AF324976</u>
		<i>tumiditarsus</i>	<i>repletoides</i>	<u>AF324962</u>	<u>AF324978</u>

Note. Newly obtained *amd* and *Ddc* sequences are underlined.

(*Dorsilopha busckii* (AF293707, AF293733), *D. paulistorum* (AF293719, AF293744), *D. willistoni* (AF293730, AF293750), *D. nebulosa* (AF293717, AF293742), *D. pseudoobscura* (AF293722, AF293746), and *Scaptodrosophila lebanonensis* (AF293714, AF293739).

DNA preparation and sequencing are described in Tataronkov *et al.* (2001). Phylogenetic analyses were performed with PAUP (version 4.0b1 for Macintosh; Swofford, 1998). We present trees only from the maximum parsimony (MP) analyses, but data sets were also analyzed with neighbor-joining (NJ) and maximum-likelihood (ML, HKY-gamma substitution model) methods.

Prior to reconstructing the phylogenetic relationships among species groups of the *virilis-repleta* radiation, we determined whether a set of representative species form a monophyletic cluster. This is achieved by analyzing the sequences listed in Table 1, together with 18 previously studied sequences of *amd* and *Ddc* encompassing higher-level Drosophilid diversity (Tataronkov *et al.*, 1999a, 2001). With the exception of *D. repletoides* (*tumiditarsus* species group), all representative species of the radiation form a well-defined monophyletic cluster on MP and NJ trees using *Ddc* and *amd* sequences separately or combined (when using *Ddc* only, *D. immigrans* clusters with *D. polychaeta*, but bootstrap support for this relationship is low). The Hawaiian drosophilids consistently appear as the closest sister-group to the *virilis-repleta* radiation, although with low bootstrap support. The close

relationship of the two lineages is also corroborated by previous studies using several genes (Russo *et al.*, 1995; Tamura *et al.*, 1995; Remsen and DeSalle, 1998; Katoh *et al.*, 2000; Tataronkov *et al.*, 1999a, 2001). Therefore, the Hawaiian drosophilids are used as outgroup in the analyses that follow. Use of several other taxa as outgroups leads to similar conclusions about the relationships within the *virilis-repleta* cluster. *Drosophila repletoides* (*tumiditarsus* group) is not a member of the clade that comprises all other species of the *virilis-repleta* radiation, but we cannot reject the possibility that it may be an early offshoot of the radiation, because the branch separating it from the cluster of the Hawaiian drosophilids plus the *virilis-repleta* species is not strongly supported. This matter could be resolved with additional gene sequences. In order to address the issue of monophyly of the radiation thoroughly, future studies should also include more taxa. In the present study we choose not to consider *D. repletoides* (and the *tumiditarsus* group it represents) as part of the *virilis-repleta* clade.

Phylogenetic relationships among 19 species of the *virilis-repleta* radiation, determined by maximum parsimony analyses, are presented in Figs. 1 A–C, separately for *amd*, *Ddc*, and *amd* + *Ddc* combined. Except for a discrepancy in the position of *D. virilis*, which is discussed below, the results of the MP analyses are consistent with NJ and ML analyses of the corresponding data sets. A summary tree, consistent with all analyses, is shown in Fig. 2.

The most derived part of the trees consists of repre-

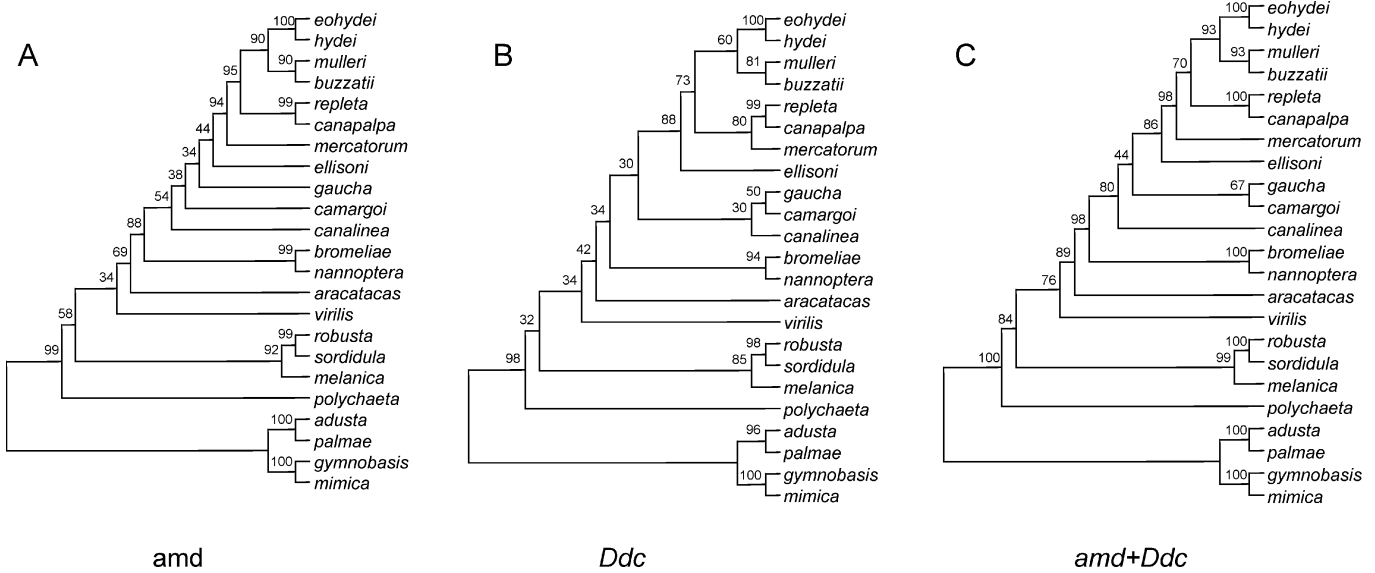


FIG. 1. Maximum parsimony (50% Majority Rule Consensus and compatible) trees for *amd* (A), *Ddc* (B), and for the two genes combined (C). Bootstrap values are shown above the nodes. Trees A and C are single most parsimonious trees. The tree shown in B is one of two equally parsimonious trees; in the other most parsimonious tree, the arrangement of *D. gaucha*, *D. camargoi*, and *D. canalinea* as in tree C. Trees were constructed in PAUP (version 4.0b1 for Macintosh; Swofford, 1998) using heuristic search with random taxa addition and the tree bisection-reconnection branch swapping.

sentatives of the *repleta* group (the eight species at the top of Figs. 1 and 2). *Drosophila ellisoni* (*fasciola* subgroup) is the most distant to the other subgroups of the *repleta* group in all trees, with strong bootstrap support in *amd* and combined *amd + Ddc* trees. The *hydei* and *mulleri* subgroups cluster in all trees, with strong support in *amd* and combined *amd + Ddc* trees. The placement of the *melanopalpa* and *mercatorum* subgroups is uncertain. *Ddc* supports *mercatorum* as the sister-taxon to the *melanopalpa* subgroup (bootstrap

80%, Fig. 1B), whereas *amd* strongly indicates (95%) that *melanopalpa* is closer to the *hydei* and *mulleri* cluster (Fig. 1A). In the combined tree the *melanopalpa* and *mercatorum* subgroups are arranged as in the *amd* tree, but bootstrap support for the closer relationship of the *melanopalpa* subgroup to the *hydei* and *mulleri* cluster becomes less robust (70%). This decrease in bootstrap support reflects the conflicting signals in the *amd* and *Ddc* data sets. The Incongruence Length Difference test (Farris *et al.*, 1994), conducted with eight species of the *repleta* group, detects significant heterogeneity between the two data sets ($P = 0.026$). A possible reason for the conflict between *amd* and *Ddc* is lineage sorting, a process that may cause discrepancies between gene trees and species trees (Maddison, 1997). The phylogenetic relationships of the *melanopalpa* and *mercatorum* subgroups are shown as polytomy 1 on the summary tree (Fig. 2)

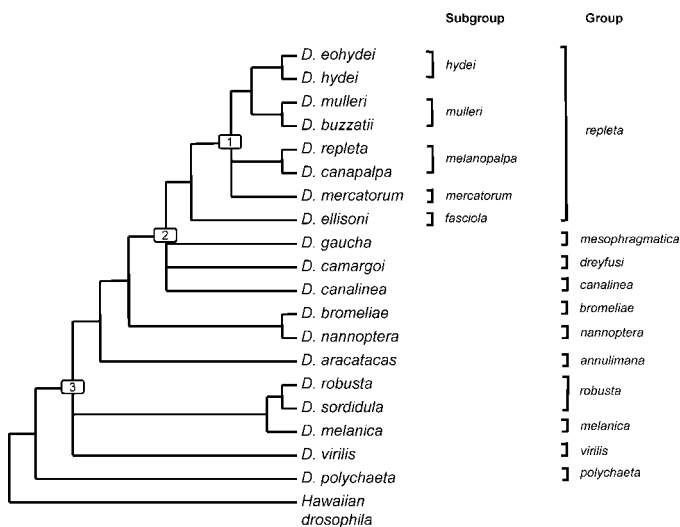


FIG. 2. Summary tree showing the phylogenetic relationships of species groups and subgroups in the *virilis-repleta* radiation. Polytomies 1, 2, and 3 are discussed in the text.

In Throckmorton's (1975) scheme the *melanopalpa* and *mercatorum* subgroups are sister taxa, while the relationships of the other subgroups of the *repleta* group are not resolved. The *mercatorum* and *melanopalpa* subgroups are also sister taxa according to chromosomal rearrangements (Wasserman, 1982, 1992; Powell, 1997). Wasserman also suggests that the *mulleri* and *fasciola* subgroups are sister taxa. This suggestion disagrees with our results, which strongly indicate that *D. ellisoni* (*fasciola* subgroup) is the earliest offshoot in the *repleta* group.

Drosophila camargoi (*dreyfusi* group), *D. gaucha* (*mesophragmatica*), and *D. canalinea* (*canalineae*) are

the closest groups to the *repleta* group. These four groups were included by Throckmorton (1975) in the *repleta* radiation. *Drosophila gaucha* and *D. camargoi* appear to be closer to each other than they are to *D. canalinea* in all analyses (Figs. 1A–C). It is not clear, however, whether the three groups are monophyletic or paraphyletic relative to the species of the *repleta* group. In any case, our results do not support Throckmorton's hypothesis that *canalinea* and *dreyfusi* groups are sister groups. The relationships among the four groups of the *repleta* radiation should be considered as unresolved due to insufficient phylogenetic signal in the data (polytomy 2 in Fig. 2).

Durando *et al.* (2000) have recently suggested that the *repleta* species group is not monophyletic, which seemingly contradicts our results. However, these authors cautioned that their inference "may be result of poor resolution at the base of the tree." Indeed, with the exception of the *mulleri* and *buzzatii* species complexes, which form a well-resolved clade, other species complexes, subgroups, and three groups of the *repleta* radiation are depicted in Durando *et al.* (2000) as a polytomy. Our analysis considerably resolves this polytomy by showing that (i) the *hydei* subgroup is closely related to the clade of *mulleri* and *buzzatii* complexes, (ii) the *melanopalpa* and *mercatorum* subgroups are more distant to the above mentioned taxa, and (iii) the *fasciola* subgroup is the early offshoot in the *repleta* group. Moreover, our analysis also clearly shows division between the subgroups of the *repleta* group and the *mesophragmatica*, *dreyfusi*, and *canalinea* groups. Although this is the traditional morphologic division, previous molecular studies (Durando *et al.*, 2000; Pél-andakis and Solignac, 1993) suggested different, although poorly resolved relationships. Despite the relatively good resolution obtained in our study, we note here that the monophyly of the *repleta* radiation, and of the *repleta* group within it, requires further verification by including more taxa, particularly from the highly heterogeneous *mulleri* species subgroup (Durando *et al.*, 2000).

The *bromeliae* and *nannoptera* groups are closely related, and they are the closest lineage to the *repleta* radiation (bootstrap 98% on the *amd* + *Ddc* MP tree). *Drosophila araccatacas* (*annulimana* group) appears to share the most recent common ancestor with the species discussed previously. However, bootstrap support for this cluster is only 89% on the combined MP tree.

The *robusta* (*D. robusta* and *D. sordidula*) and *melanica* groups are closely related. The position of *D. virilis* is ambiguous. On the MP and ML trees, it belongs to the lineage encompassing the *repleta* radiation, and the *bromeliae*, *nannoptera*, and *annulimana* groups, but bootstrap support for this cluster is only 76% in the MP tree. However, in the NJ tree, *D. virilis* joins the cluster of *robusta* and *melanica* groups with high support (96%). The topologies of the two trees are not

different according to the Kishino-Hasegawa test (1989). Apparently, this outcome of the Kishino-Hasegawa test is due to low support for the *D. virilis* position in the MP tree. Considering that support for the position of *D. virilis* in the MP tree is not high, we favor the view that *D. virilis* is a sister taxon to the *robusta* and *melanica* groups. However, because the alternative topologies do not differ according to KH tests under both ML and MP criteria, we take a conservative position by considering the location of *D. virilis* as unresolved (polytomy 3 in Fig. 2). The position of the *virilis* group relative to the other species groups is ambiguous in other molecular studies as well. Thus, in the analysis of four genes by Remsen and DeSalle (1998) *D. virilis* is closer to *D. repleta* than it is to *D. melanica* and *D. robusta*, whereas in the *Adh* analysis by Katoh *et al.* (2000), the *virilis* group species are closer to *D. sordidula* (*robusta* group) than to the *repleta* group species.

This study is one of a few attempts to sort out the huge diversity of the *virilis-repleta* radiation using DNA sequences. The two genes we have used, *amd*, and *Ddc*, are quite informative about *Drosophila* relationships at this level of divergence, resulting in a fairly resolved phylogenetic tree.

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REFERENCES

- Durando, C. M., Baker, R. H., Etges, W. J., Heed, W. B., Wasserman, M., and DeSalle, R. (2000). Phylogenetic analysis of the *repleta* species group of the genus *Drosophila* using multiple sources of characters. *Mol. Phylogenet. Evol.* **16**: 296–307.
- Eveleth, D. D., Gietz, R. D., Spencer, C. A., Nargang, F. E., Hodgetts, R. B., and Marsh, J. L. (1986). Sequence and structure of the dopa decarboxylase gene of *Drosophila*: Evidence for novel RNA splicing variants. *EMBO J.* **5**: 2663–2672.
- Farris, J. S., Källersjö, M., Kluge, A. G., and Bult, C. (1994). Testing significance of incongruence. *Cladistics* **10**: 315–319.
- Hartl, D. L., and Lozovskaya, E. R. (1995). "The *Drosophila* Genome Map: A Practical Guide." R. G. Landes, Austin, TX.
- Katoh, T., Tamura, K., and Aotsuka, T. (2000). Phylogenetic position of the subgenus *Lordiphosa* of the genus *Drosophila* (Diptera: Drosophilidae) inferred from alcohol dehydrogenase (*Adh*) gene sequences. *J. Mol. Evol.* **51**: 122–130.
- Kishino, H., and Hasegawa, M. (1989). Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data, and the branching order in Hominoidea. *J. Mol. Evol.* **29**: 170–179.
- Maddison, W. P. (1997). Gene trees in species trees. *Syst. Biol.* **46**: 523–536.
- Marsh, J. L., Erfle, M. P., and Leeds, C. A. (1986). Molecular localization, developmental expression and nucleotide sequence of the

- alpha-methyl dopa hypersensitive gene of *Drosophila*. *Genetics* **114**: 453–467.
- Nurminsky, D. I., Moriyama, E. N., Lozovskaya, E. R., and Hartl, D. L. (1996). Molecular phylogeny and genome evolution in the *Drosophila virilis* species group: Duplications of the alcohol dehydrogenase gene. *Mol. Biol. Evol.* **13**: 132–149.
- Patterson, J. T., and Stone, W. S. (1952). "Evolution in the Genus *Drosophila*." Macmillan Co., New York.
- Pélandakis, M., and Solignac, M. (1993). Molecular phylogeny of *Drosophila* based on ribosomal RNA sequences. *J. Mol. Evol.* **37**: 525–543.
- Powell, J. R. (1997). "Progress and Prospects in Evolutionary Biology: The *Drosophila* Model." Oxford University Press, New York.
- Remsen, J., and DeSalle, R. (1998). Character congruence of multiple data partitions and the origin of the Hawaiian Drosophilidae. *Mol. Phylogenet. Evol.* **9**: 225–235.
- Russo C. A. M., Takezaki, N., and Nei, M (1995). Molecular phylogeny and divergence times of drosophilid species. *Mol. Biol. Evol.* **12**: 391–404.
- Swofford, D. (1998). "PAUP*: Phylogenetic Analysis Using Parsimony (*and Other Methods)." Sinauer, Sunderland, MA.
- Tamura, K., Toba, G., Park, J., and Aotsuka, T. (1995). Origin of Hawaiian drosophilids inferred from alcohol dehydrogenase gene sequences. *In*: "Current Topics on Molecular Evolution. Proceedings of the US–Japan Workshop, Hayama, Japan. 25–27 August 1995" (M. Nei and N. Takahata, Eds.), pp. 9–18. Pennsylvania State University Press, University Park, PA; Graduate School for Advanced Studies, Hayama, Japan.
- Tatarenkov, A., Kwiatowski, J., Skarecky, D., Barrio, E., and Ayala, F. J. (1999a). On the evolution of *Dopa decarboxylase* (*Ddc*) and *Drosophila* systematics. *J. Mol. Evol.* **48**: 445–462.
- Tatarenkov, A., Sáez, A. G., and Ayala, F. J. (1999b). A compact gene cluster in *Drosophila*: The unrelated *Cs* gene is compressed between duplicated *amd* and *Ddc*. *Gene* **231**: 111–120.
- Tatarenkov A., Žurovcová M., and Ayala F. J. (2001). *Ddc* and *amd* sequences resolve phylogenetic relationships of *Drosophila*. *Mol. Phylogenet. Evol.* **20**: 321–325.
- Throckmorton, L. H. (1962). The problem of phylogeny in the genus *Drosophila*. *Stud. Genet. II Univ. Texas Publ.* **6205**: 207–343.
- Throckmorton, L. H. (1975). The phylogeny, ecology, and geography of *Drosophila*. *In*: "Handbook of Genetics" (R. C. King, Ed.), pp. 421–459. Plenum, New York.
- Wasserman, M. (1982). Cytological evolution in the *Drosophila repleta* species group. *In* "Ecological Genetics and Evolution: The Cactus–Yeast–*Drosophila* Model System" (J. S. F. Barker and W. T. Starmer, Eds.), pp. 49–64. Academic Press, New York.
- Wasserman, M. (1992). Cytological evolution of the *Drosophila repleta* species group. *In* "*Drosophila* Inversion Polymorphism" (C. B. Krimbas and J. R. Powell, Eds.), pp. 455–552. CRC Press, Boca Raton, FL.
- Yoon, J. (1996). Stock list: National *Drosophila* Species Resource Center. *Drosophila Information Service* **77**: 1–44.