# The *ercepeae* Complex: New Cases of Insular Speciation within the *Drosophila ananassae* Species Subgroup (*melanogaster* Group) and Descriptions of Two New Species (Diptera: Drosophilidae)

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**ABSTRACT** Two new species of the *Drosophila ananassae* subgroup are described. *D. comorensis* Tsacas sp. n. and *D. merina* Tsacas sp. n. are endemic to the Indian Ocean islands Comores and Madagascar, respectively. Comparisons with 2 other species, also endemic to Indian Ocean islands and belonging to the *D. ananassae* subgroup, *D. ercepeae* Tsacas & David from La Réunion and *D. vallismaia* Tsacas from Seychelles, lead to the definition of a 3rd complex, *ercepeae*, within this subgroup. Relationships among the 4 species of the *ercepeae* complex are based on morphological data (the only data available for *D. comorensis*), mitotic and polytene chromosome analyses, behavioral interactions, interspecific hybridizations, chemical analyses of cuticular hydrocarbons, and amylase polymorphism. Within this complex, *D. ercepeae* and *D. merina* are distinguished from *D. vallismaia* and *D. comorensis*. All characters show close affinities between *D. ercepeae* and *D. merina*. The geographic distribution of the species of the 3 complexes of the *D. ananassae* subgroup is also discussed.

**KEY WORDS** *Drosophila melanogaster* group, *ananassae* subgroup, insular speciation, species complex

A NUMBER OF significant studies of genetic divergence and speciation in island populations of *Drosophila* have been published. Certainly, the grandest example of this is the detailed series of studies of the spectacular array of *Drosophila* species native to the Hawaiian Islands. Other island groups and *Drosophila* species have also been investigated by Stone et al. (1966). Among these studies are those on *D. ananassae* Doleschall populations from islands of central and western Pacific Ocean. Futch (1966) demonstrated cytologically that nearly indistinguishable light and dark *D. ananassae*-like populations found on the American Samoan island of Tutuila were isolated sexually. Bock and Wheeler (1972) classified the light *ananassae* form as a new species, *D. pallidosa* Bock & Wheeler. The reproductive isolation between *D. pallidosa* and *D. ananassae*, which coexist on Tutuila, was reported by Futch (1973). But, under laboratory conditions, Tobari (1993) found that this reproductive isolation was not complete.

*Drosophila ananassae* was first described by Doleschall (1858) from Ambon (Ambonia), a small island off the southwestern tip of Ceram, Indonesia. It is now well known as a domestic species with a quasicosmopolitan distribution. *D. ananassae* is widely distributed in the Oriental region, not only on the continents, but also on numerous islands (Tobari 1993).

The *ananassae* subgroup belongs to the *melanogaster* group, which includes 12 subgroups (Toda 1991). The relationships among the different species subgroups are still not well understood. Ashburner et al. (1984) made an attempt to integrate the chromosomal and morphological data to give an overall hypothesis of the relationships among the subgroups. They recognized 3 central lines within the *melanogaster* group. *D. ananassae* subgroup constitutes the 1st of the central lines, the 2nd consists of the *montium* subgroup, and the 3rd is composed of the *suzukii, takahashii, ficusphila, melanogaster, elegans,* and *eugracilis* subgroups. The remaining subgroups may be separated from these lines.

The *ananassae* subgroup includes 22 species. Within this subgroup, 2 species complexes, *ananassae* (8 species) and *bipectinata* (4 species), were distinguished on the basis of the structure of their male genitalia (Bock 1971, Bock and Wheeler 1972). A 3rd complex, named *ercepeae*, will be de-

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scribed in this article. The remaining species of the *ananassae* subgroup are not members of these complexes. This article provides diagnoses of, and descriptive and biogeographical data on, the 4 species here grouped as the *ercepeae* complex. All these species are insular endemics in the Indian Ocean: *D. ercepeae* Tsacas & David, living in La Réunion; *D. vallismaia* Tsacas, living in Seychelles; *D. merina* Tsacas sp. n., living in Madagascar; and *D. comorensis* Tsacas sp. n., living in Comores. Analyses of mitotic and polytene chromosomes, amylase variants, behavioral interactions, hybridizations, and cuticular hydrocarbons are presented. This study, then, gathers together different facets of systematics and investigates the phylogenetic relationships among the species of the complex, and tries to determine their position within the *ananassae* subgroup.

## Materials and Methods

***Drosophila* Strains.** All species originated from Indian Ocean islands. *D. ercepeae* (stock No. 164-14 of Populations, Génétique et Evolution [PGE] laboratory, Centre National de le Recherche Scientifique [CNRS]) (Tsacas and David 1975) was initiated with flies collected in La Réunion by J. David (in 1973); *D. vallismaia* (stock No. 206-11 of PGE laboratory, CNRS) (Tsacas 1984) in Seychelles by J. David and L. Tsacas (in 1977); *D. merina* (stock No. 290-1 of PGE laboratory, CNRS) in Madagascar by S. Aulard, J. David, and S. F. McEvey (in 1987); *D. comorensis* in Comores (L. Matile leg, 1974). Only dead specimens were available for this last species, so only morphological analyses were undertaken. All living stocks were maintained at 20°C on cornmeal medium supplemented with Carolina instant *Drosophila* medium.

**Behavioral Analyses.** Flies were sexed under light ether anesthesia and maintained in glass vials with food medium. Males were stored individually, and females were kept in groups of 5. Observations were performed in glass cells (30 mm diameter, 3 mm high) without food. A single male was first aspirated and 1 female was introduced 1 min later. The behavior of the flies was then observed.

*Copulation Kinetics.* For every species, isolated pairs were observed for 1 h. These tests were performed with flies whose ages corresponded to their highest sexual activity. Numbers of copulations were recorded for consecutive periods of 5 min and then cumulated.

*Intraspecific Courtships and Matings.* For each species, 10 pairs of virgin flies, 4, 7, or 11 d old, were individually observed for 1 h. The number of courtships and matings was recorded.

*Interspecific Matings.* Pairs (55–68) of each of the 9 possible combinations of the 3 parental species *D. ercepeae, D. vallismaia,* and *D. merina* were observed during 60 min. Courtship and mating numbers were recorded.

*Interspecific Hybridizations.* Hybridizations were done under no choice conditions using 10 males and 10 females per tube. All crosses were done at 20°C on standard cornmeal medium supplemented with Carolina food. To ensure that all flies were virgin, old pupae were isolated in separate tubes until the adults emerged. Different sets of males and females (10 × 10) were kept in separate tubes with food. All crosses were done when the flies were 8 d old and each cross was transferred twice a week into a fresh vial. Each cross was repeated at least 5 times (more if the results were at all variable).

**Chemical Analyses of Cuticular Hydrocarbons.** Cuticular extracts were obtained by submerging single flies into 50 μl hexane for 2 min and shaking for 1 min. They were then analyzed on a gas chromatograph (GC) Girdel 300 equipped with a capillar Cp sil 5 column (25 m by 0.34 mm). GC-MS analysis (GC coupled with mass spectrometry) was carried out on a Nermag R10-10 (see Antony et al. 1985), and electron impact (70 ev) with similar conditions for GC. The structure was deduced from the molecular mass and the fragmentation pattern.

**Chromosomal Analyses.** *Mitotic Chromosomes.* Mitotic metaphase or late prophase chromosomes were prepared from the brains of 3rd instars. After dissection in a Ringer's solution, the tissue was treated with colchicine (1 mg/ml) for 1 h and then hypotonically with 1% sodium citrate for 10 min. The chromosomes were fixed in ethanol : glacial acetic acid (3 : 1, vol : vol) for 3–5 min. The fixed tissue was then transferred to a drop of 60% acetic acid for 10 min. Finally, the slide was air dried on a hotplate (45°C). The chromosomes were stained with 3% Giemsa. *Polytene Chromosomes.* Salivary gland polytene chromosomes from 3rd instars were dissected in a Ringer's solution. The glands were first fixed for 1 min in 45% acetic acid, then for 3 min in lactic acid : water : acetic acid (1:2:3, vol : vol : vol) and stained for 1–2 min in lacto-aceto-orcein (2% orcein in 1:1:1, lactic acid : acetic acid : water).

**Electrophoretic Analyses.** Amylase polymorphism was assayed by polyacrylamide gel electrophoresis with 0.1 M Tris-borate buffer, pH 8.9. After electrophoresis, the gels were first incubated in a starch solution and stained with potassium iodine (Daïnou et al. 1987). The amylase variants were compared with a *D. ananassae* reference and numbered according to their mobility. Increasing numbers correspond to decreasing mobility (Da Lage et al. 1989).

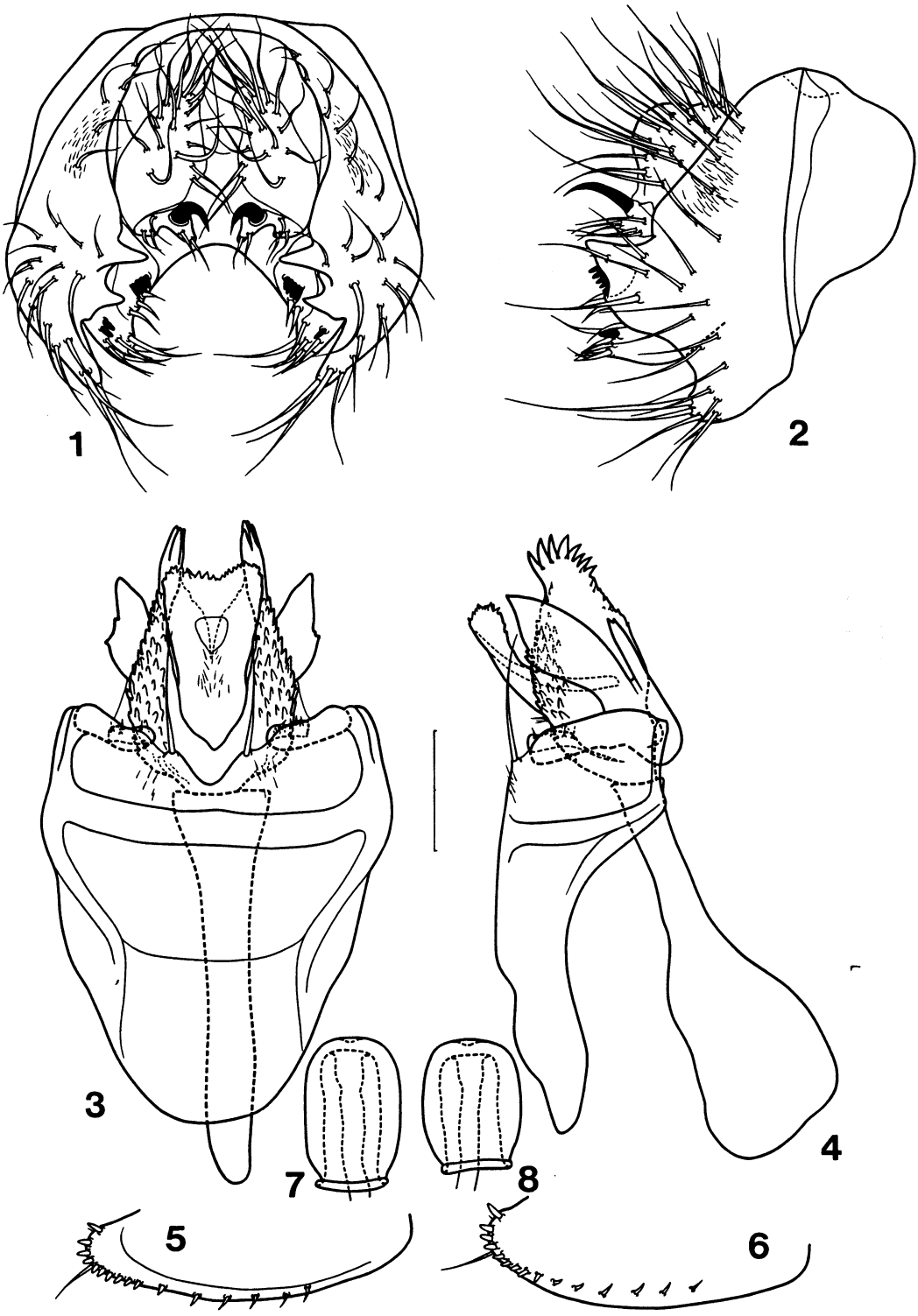
## Results

### *Drosophila* (*Sophophora*) *merina* Tsacas sp. n.

(Figs. 1–8)

*ercepeae*-like, Da Lage et al. 1989: 71

**Diagnosis.** *D. merina* has male terminalia closely resembling those of *D. ercepeae.* However, dis-




**Figs. 1–8.** *D. merina* sp. n. Terminalia of holotype male and female. (1) Epandrium caudal view. (2) *id.*, Lateral view. (3) Hypandrium and aedeagus ventral view. (4) *id.*, Lateral view. (5 and 6) Ovipositors. (7 and 8) Spermathecae.

tinct differences in the aedeagus and parameres provide unequivocal means for identifying this species.

**Male.** *Head.* Frons slightly brownish with a lighter transverse stripe above the antennae sometimes extending by a more or less diffuse extension to the ocellar-triangle. *Indices.* (Head width, hw): (frontal width, fw) = 2.1; (frontal width, fw):(frontal length, fl) = 1.4; (orbital setae, or) or1:or3 = 0.9; or1:or2 = 1.9. Postverticals parallel or weakly convergent (rarely slightly crossed). *Antennae.* Pedicel and flagellum yellowish brown, arista with 5–6 dorsal branches and 3 ventral branches (rarely 4) plus a terminal fork. Face grayish, shiny, carina and epistome whitish. Two vibrissae. Palpus reddish with a long preapical bristle, with fine setulae. Cheeks pale, narrow, shiny. Eyes tannish red, eye: cheek ratio = 14. *Thorax.* Scutum reddish brown, shiny, with 8 rows of acrostichal setulae and 2 pairs of dorsomedial setae. Scutellum concolorous with scutum, basal scutellar bristles slightly convergent, apicals crossed, ratio of their lengths b:a = 0.9. Pleura paler than scutum, nonuniform, sterno-index = 0.5, a 3rd intermediate katepisternal seta slender, about half the anterior one. *Legs.* Slightly brown, anterior coxa paler, preapicals on all tibiae, apicals on fore and midtibiae, minute on fore tibiae. First and 2nd tarsomeres of forelegs with some small spines apically, these arranged in transverse rows (sex combs). One sex comb with 3–4 spines on 1st tarsomere, and 2 sex combs with 2–3 spines each on 2nd tarsomere (sometimes 4 spines on distal sex comb). *Wings.* Slightly brownish and iridescent, veins tan. *Indices.* L:l = 2.5; C-index = 2.1; 4v-index = 2.3; 4c-index = 1.3; 5x-index = 2.0; ac-index = 2.6; C3 fringe = 60%. Halteres concolorous with pleura. *Abdomen.* Paler than thorax, yellowish, 2nd and 3rd tergites (tergites II and III) with narrow brown caudal band, tergites IV and V with diffuse caudal band, tergite VI without band. *Terminalia.* Pale, closely resembling those of *D. ercepeae.* Ventral margin of the epandrium with triangular, not serrate, expansion. Posterior parameres with a bifid shape of the anterior lobe of the posterior branch. *Aedeagus.* Basiphallus bifid, composed of 2 plates strongly serrate apically (8–9 spines) and ventrally covered by spinules, distiphallus nonbifid, erected from these plates, broad ventrally, with apical margin almost straight and serrate (Figs. 1–4).

**Female.** Similar to male, tergites with black, shiny caudal band, tergite IV with narrower caudal band. *Indices.* Hw:fw = 2.1; fw:fl = 1.4; or1:or3 = 0.8; or1:or2 = 1.9; eye : cheek ratio = 13.5. Scutellar bristles b:a = 0.9; sterno-index = 0.5; wings L:l = 2.5; C-index = 2.2; 4v-index = 2.4; 4c-index = 1.3; 5x-index = 1.9; ac-index = 2.5; C3 fringe = 57%. Ovipositor and spermathecae, showing slight variability, similar to those of *D. ercepeae* (Figs. 5–8).

Male body length: 2.7 mm; wing length: 2.4 mm.

Female body length: 2.8 mm; wing length: 2.5 mm.

**Material Examined.** HOLOTYPE, ♂, MADAGASCAR: Mandraka, 11/13–X–1987 (S. Aulard, J. R. David, and S. F. McEvey). PARATYPES, 25 ♂ and 20 ♀, same data as holotype flies from the strain No. 290.1 of PGE-CNRS laboratory. (Museum National d'Histoire Naturelle de Paris).

**Distribution.** MADAGASCAR, Mandraka.

**Etymology.** Merina, name of the main human population of Madagascar.

### *Drosophila (Sophophora) comorensis* Tsacas sp. n.
(Figs. 9–16)

**Diagnosis.** Pale, close to *D. ananassae* Doleschall, but differing by tergites with narrow caudal bands. Close to *D. vallismaia* by its terminalia. Sex combs of male anterior tarsa difficult to detect with a row of 4–5 spines on 1st tarsomere, 2–3 on 2nd. Except for these features, the external morphology of *D. comorensis* does not differ from that of *D. ananassae.*

We report here only values of the different indices and the terminalia description.

**Male.** *Terminalia.* Pale. Ventral margin of the epandrium with wide expansion whose serrate edge points to superior margin. Parameres simple, elongate, apex ventrally curved, dorsal edge weakly serrate, basal expansion joined to novasternum. *Aedeagus.* Basiphallus bifid, lateral plates with 4–5 apical spines and numerous smaller spines ventrally, distiphallus membranous, nonbifid (Figs. 9–14). *Indices.* Hw:fw = 2.0; fw:fl = 1.4; or1:or3 = 0.8; or1:or2 = 1.7; arista with 5–6 dorsal branches and 3 ventral branches plus terminal fork; eye : cheek ratio = 14; sterno-index = 0.7; wings L:l = 2.4; C-index = 1.9; 4v-index = 2.3; 4c-index = 1.4; 5x-index = 1.9; ac-index = 3.0; C3 fringe = 60%.

**Female.** Ovipositor and spermathecae identical to those of *D. vallismaia,* with more short setae (≈18) on ovipositor (Figs. 15–16). *Indices.* Hw:fw = 2.0; fw:fl = 1.5; or1:or3 = 0.8; or1:or2 = 2.0; arista with 5 dorsal branches and 3 ventral branches plus terminal fork; eye:cheek ratio = 13; sterno-index = 0.7; wings L:l = 2.3; C-index = 2.1; 4v-index = 2.4; 4c-index = 1.4; 5x-index = 1.9; ac-index = 2.9; C3 fringe = 67%.

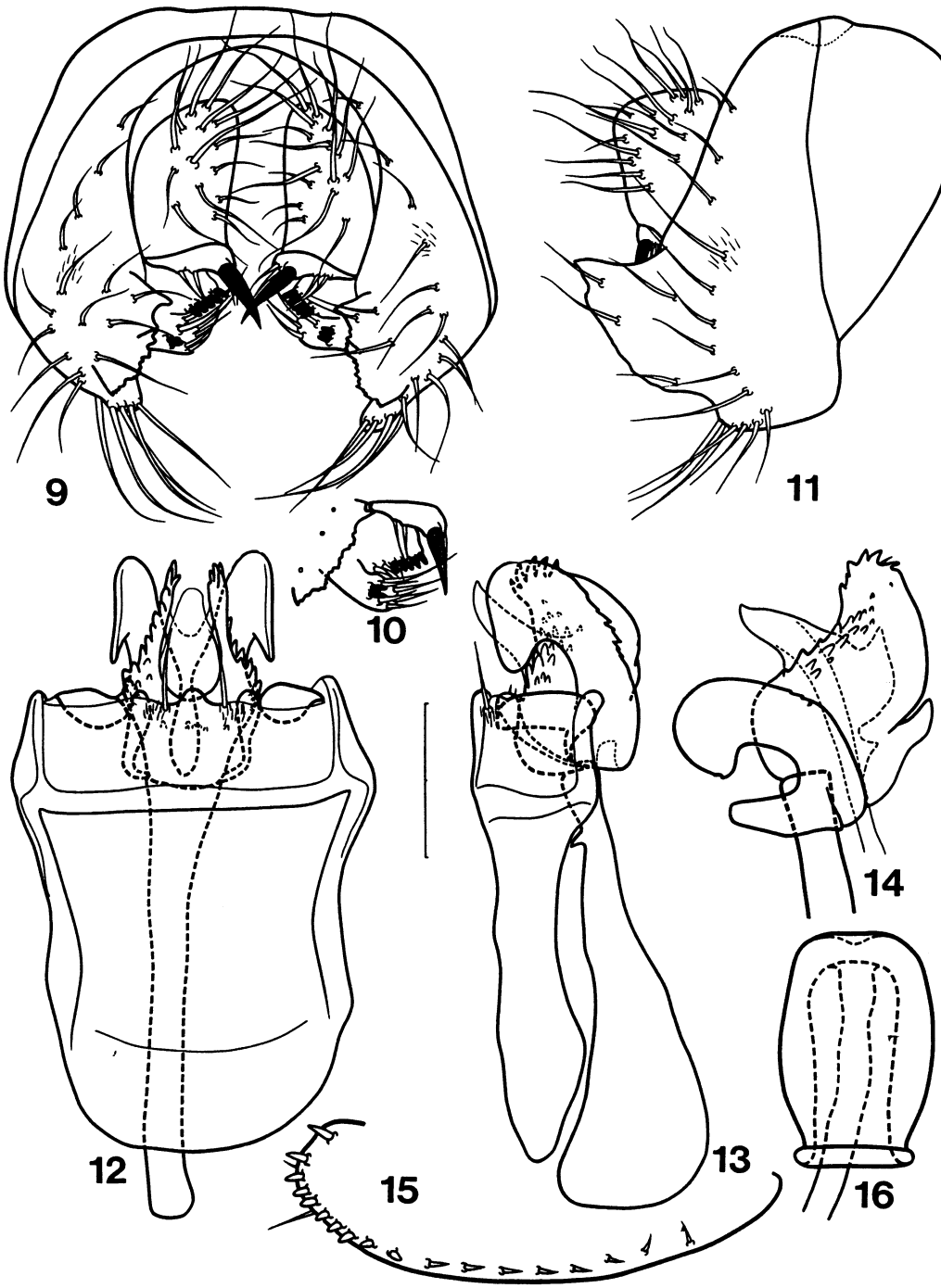
Male body length: 2.7 mm; wing length: 2.2 mm.

Female body length: 3.3 mm; wing length: 2.6 mm.

**Material Examined.** HOLOTYPE, ♂, COMORES: Grande Comore, La Grille (Guiri), 850–900 m, 10–I–1974 (L. Matile). PARATYPES, 1 ♂ and 1 ♀, same data as holotype; 2 ♂, Mohéli, Djoumandounia, 100–150 m., 29–XI–1973 (L. Matile). (Museum National d'Histoire Naturelle de Paris).

**Distribution.** COMORES: Grande Comore and Mohéli.

**Etymology.** The name indicates the origin of the type of the species.

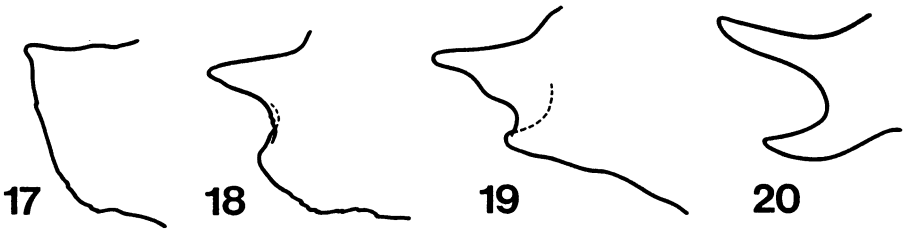



**Figs. 9–16.** *D. comorensis* sp. n. Terminalia of holotype male and female. (9) Epandrium caudal view. (10) Surstylus. (11) Epandrium lateral view. (12) Hypandrium and aedeagus ventral view. (13 and 14) Aedeagus lateral view. (15) Ovipositor. (16) Spermatheca.

### The *ananassae* Subgroup Complexes

Until the discovery of the *ercepeae* complex, species of the *ananassae* subgroup were divided into 2 complexes, mainly characterized by the structure of their aedeagus (Lemeunier et al. 1986):

***ananassae* Complex.** Aedeagus nonbifid (Kaneshiro and Wheeler 1970, Bock 1971): *D. ananassae* Doleschall, *D. atripex* Bock & Wheeler, *D. lachaisei* Tsacas, *D. monieri* McEvey & Tsacas, *D. nesoetes* Bock & Wheeler, *D. ochrogaster* Chassag-




**Figs. 17–20.** Expansion of the posterior edge of the epandrium. (17) *D. vallismaia.* (18) *D. comorensis.* (19) *D. ercepeae.* (20) *D. merina.*

nard in Chassagnard and Groseille (1992), *D. pallidosa* Bock & Wheeler, and *D. phaeopleura* Bock & Wheeler.

***bipectinata* Complex.** Aedeagus bifid (Kaneshiro and Wheeler 1970, Bock 1971): *D. bipectinata* Duda, *D. malerkotliana* Parshad & Paika, *D. parabipectinata* Bock, and *D. pseudoananassae* Bock.

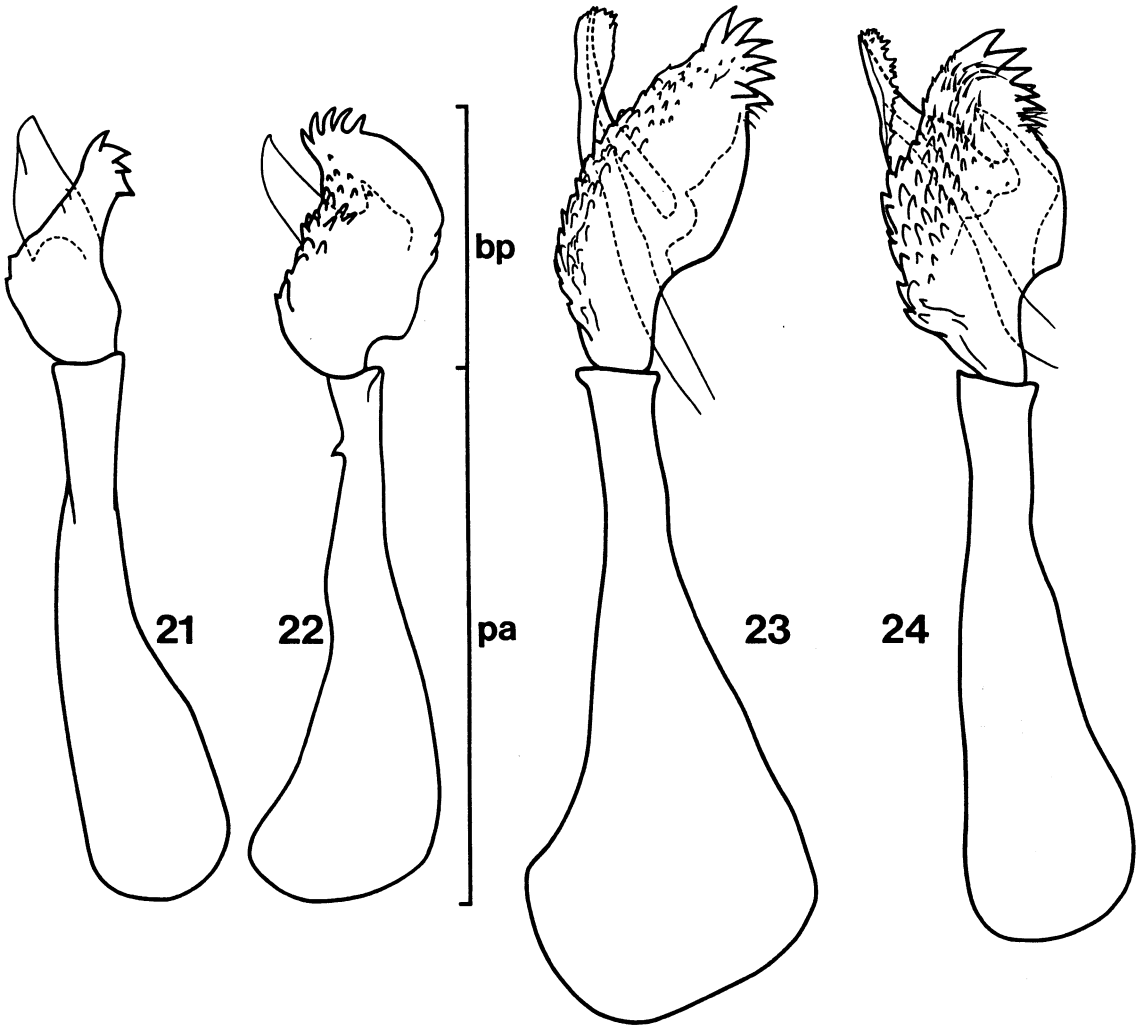
***ercepeae* Complex.** The 4 species, *D. comorensis* Tsacas sp. n., *D. ercepeae* Tsacas & David, *D. merina* Tsacas sp. n., and *D. vallismaia* Tsacas, all possess terminalia whose exclusive morphological characters separate them from members of the other complexes. Tsacas (1984) already pointed out the similarities between *D. ercepeae* and *D. vallismaia.*

The *ercepeae* complex possesses all the characteristics of the *ananassae* subgroup, but differs from the 2 other complexes by the aedeagus structure. It has a distinct basiphallus with 2 plates fused in their basal half and a distiphallus arising from these plates. Such a structure is intermediate between the nonbifid aedeagus of the *ananassae* complex and the aedeagus of the *bipectinata* complex composed of 2 plates fused at their bases, but without an individualized distiphallus. The *ercepeae* complex constitutes the 3rd complex of the *ananassae* subgroup. With regard to their external morphology, the 4 species are very similar but they may be distinguished by 4 characters of the male terminalia.

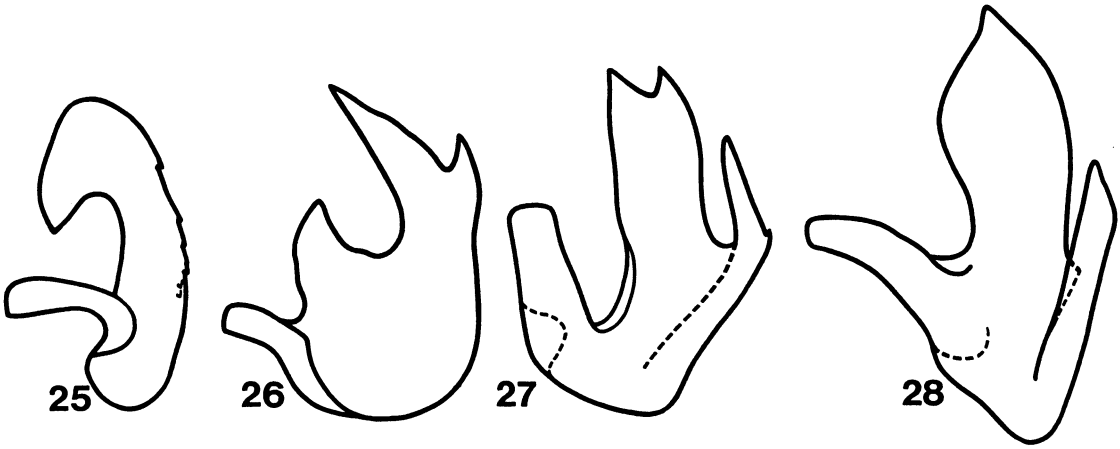
*Epandrium.* The shape of the expansion of the posterior edge is characteristic of every species. These expansions may be ordered linearly, from the simplest to the most complicated (Figs. 17–20): from *D. comorensis,* with an almost straight expansion; to *D. ercepeae* and *D. merina,* with slightly pointed expansions; and to *D. vallismaia,* whose expansions have 2 distinct lobes. *Aedeagus.* The aedeagus of the *ercepeae* complex is distinctive among the *ananassae* subgroup species. The distinct basiphallus has the 2 plates fused in their basal half and a distiphallus erected from these plates. As previously mentioned, this structure is intermediate between the nonbifid aedeagus of the *ananassae* complex and the aedeagus of the *bipectinata* complex composed of 2 plates fused at their bases, and without an individualized distiphallus. Within the *ercepeae* complex, there are also differences: *D. vallismaia* has a distiphallus and a basiphallus without ornamentation, with few teeth; *D. comorensis* has a simple distiphallus, a slightly ornamented basiphallus, and slightly more teeth; *D. merina* and *D. ercepeae* have increasing ornamentation on basiphallus and distiphallus (Figs. 21–24). The aedeagus of *D. comorensis* also differs from that of *D. vallismaia* by the shape of its dorsal margin—convex in *D. comorensis, D. merina,* and *D. ercepeae,* and concave in *D. vallismaia. Phallapodeme.* The evolution of the phallapodeme shape (Figs. 21–24) seems irregular, whereas its relative size, compared with that of the basiphallus (phallasomal index [Okada 1955]), increases regularly: *D. vallismaia* = 0.42, *D. comorensis* = 0.5, *D. merina* = 0.59, *D. ercepeae* = 0.63. *Parameres.* These also separate the species (Figs. 25–28). As with the epandrium, the parameres of *D. comorensis* are the simplest, with 2 branches, the paramere itself, and a ventral branch that fuses it to the epandrium. In the 3 other species, a dorsal branch lies above the ventral one. This dorsal branch is not distinct in *D. vallismaia,* but those of *D. ercepeae* and *D. merina* are well individualized.

Thus, *D. ercepeae* and *D. merina* show close similarities in most of the terminalia, although small but constant differences exist. *D. comorensis* and *D. vallismaia* are close in 3 of 4 morphological characters of the terminalia (aedeagus, phallapodeme, and posterior parameres), but they differ by the shape of the expansion of the epandrium. It is reasonable to conclude that *D. comorensis* and *D. vallismaia* differ more from each another than either does from *ercepeae* + *merina* pair.

**Chromosomal Analyses.** *Mitotic Chromosomes.* According to Hinton and Downs (1975) and Matsuda et al. (1983), the *D. ananassae* female karyotype consists of 4 pairs of V-shaped chromosomes, one of which is shorter than the other 3 (element F or chromosome 4); whereas, the male complement is a V-shaped X and a rod-shaped or a J-shaped Y chromosome, and 3 V-shaped autosomes. The *ananassae* subgroup is characterized by a metacentric X chromosome whose arms are euchromatic. This *ananassae* X must have been derived from a rod-shaped chromosome by a pericentric inversion. There is a great variation among species in the shape of element F. In nearly all




**Figs. 21–24.** Distiphallus and basiphallus. Lateral views. (21) *D. vallismaia.* (22) *D. comorensis.* (23) *D. ercepeae.* (24) *D. merina.*




**Figs. 25–28.** Parameres. Lateral views. (25) *D. comorensis.* (26) *D. vallismaia.* (27) *D. ercepeae.* (28) *D. merina.*

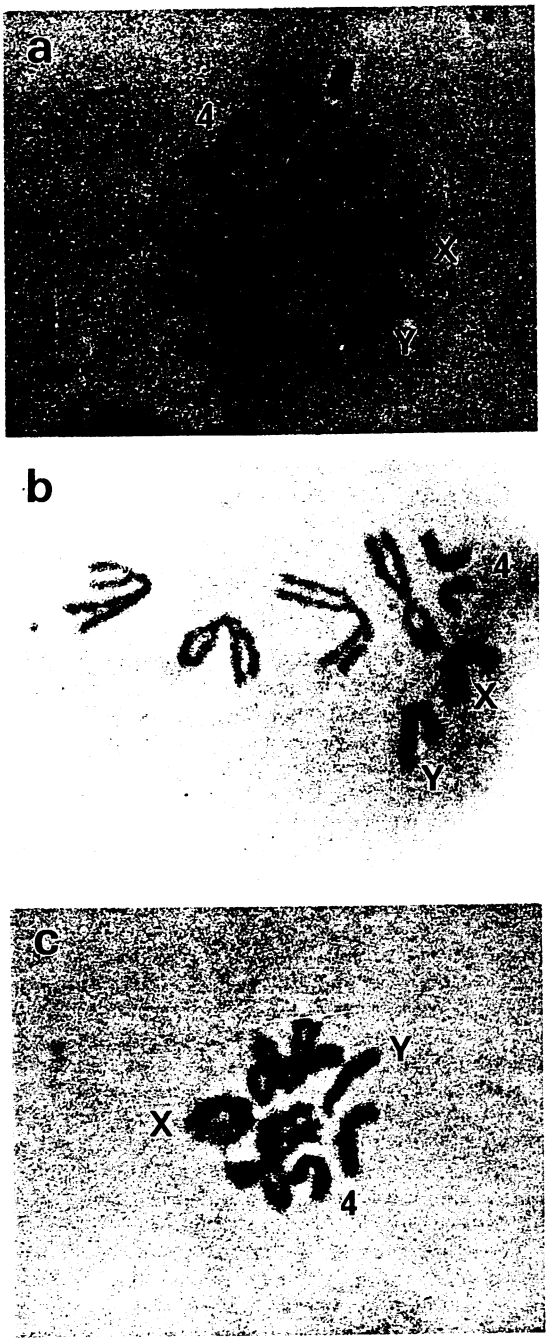



**Fig. 29.** Male karyotypes of the *ercepeae* complex. (a) *D. ercepeae.* (b) *D. merina.* (c) *D. vallismaia.*

species, it has grown from a rod by accretion of heterochromatin and is metacentric.

The karyotypes of the *ercepeae* complex (Fig. 29) agree completely with those of the *ananassae* subgroup. *D. ercepeae* and *D. merina* have almost the same karyotype, with 4 pairs of V-shaped chromosomes in the females. The male complement has a V-shaped X chromosome and a J-shaped Y chromosome in both species. The Y chromosome appears to be entirely heterochromatic. Element F is the smallest of the complement, visible as a small metacentric pair. The *D. vallismaia* karyotype is similar to those of the other species, with 2 large metacentric pairs, a metacentric X, and a J-shaped heterochromatic Y chromosome. In this species, the 4th metacentric pair is slightly longer than that of *D. ercepeae* and *D. merina,* and both arms carry subterminal constrictions and are heavily heterochromatic. All except 2 (*D. nesoetes* and *D. andamanensis*) of the 21 species of the *ananassae* subgroup whose karyotypes were examined have a similar metaphase karyotype.

The variation within the metaphase karyotypes of the *ananassae* species subgroup corresponds to the variation in the *melanogaster* species subgroup. It contrasts with the remarkable karyotypic stability of some other groups of *Drosophila,* the Hawaiian ones, for example, whose 90% of the endemic species have 6 rod and 1 dot chromosomes.

*Polytene Chromosomes.* Several reference maps of the 6 polytene chromosome arms of *D. ananassae* have been prepared since the initial observations of Kaufman (1937) and Kikkawa (1938). The most recent is that of Tobari et al. (1993), which is a revision of the photomap made by Moriwaki and Ito (1969). In *D. vallismaia, D. ercepeae,* and *D. merina* there are 6 arms extending from the chromocenter in nuclei of salivary glands cells, as in *D. ananassae.* The identification of each arm was possible because the tips closely resemble those of *D. ananassae.* So, by analogy, it was possible to ascertain 2 arms for the X chromosome (XL and XR), and 2 arms for each of the chromosomes 2 and 3 (2L, 2R, 3L, and 3R). As previously said, the 4th chromosome is completely heterochromatic and not represented in the polytene chromosomes. The polytene chromosomes of the 3 species are almost homosequential. It was possible to demonstrate the homologies because in interspecific hybrids the pairing of the homologs is almost perfect. A needed detailed comparison of the polytene chromosome banding pattern of the 3 species of the complex to that of *D. ananassae* is now in progress, and will determine the relationships between the different species belonging to the different complexes. As Bock and Wheeler (1972) have stated, there is an overall similarity in the banding pattern of the *ananassae* species complex, even if the evolution of this complex is far from clear.

*Drosophila ananassae* is one of the earliest species in which chromosomal polymorphism in natural populations has been discovered (Kaufmann 1936). Tobari et al. (1993) summarize 69 paracentric inversions. No inversion was found within the *ercepeae* complex, but we cannot conclude that there is no inversion polymorphism because only 1 strain and a few individuals of each of the 3 species were studied.

**Table 1. Percentages of pairings between species of the *ercepeae* complex leading to courtships and copulations during 1 h of observation**

| Females | Males | | | Mean |
|---|---|---|---|---|
| | *D. ercepeae* | *D. vallismaia* | *D. merina* | |
| | Courtships | | | |
| *D. ercepeae* | 13.2 | 2.9 | 31.0 | 15.7 |
| *n* | 68 | 67 | 58 | |
| *D. vallismaia* | 20.9 | 22.8 | 29.6 | 24.4 |
| *n* | 62 | 57 | 54 | |
| *D. merina* | 42.2 | 3.6 | 38.9 | 28.2 |
| *n* | 64 | 55 | 54 | |
| Mean | 25.4 | 9.8 | 33.2 | |
| | Copulations | | | |
| *D. ercepeae* | 8.8 | 0 | 0 | 2.9 |
| *n* | 68 | 67 | 58 | |
| *D. vallismaia* | 6.5 | 10.5 | 0 | 5.7 |
| *n* | 62 | 57 | 54 | |
| *D. merina* | 31.2 | 3.6 | 24.1 | 19.6 |
| *n* | 64 | 55 | 54 | |
| Mean | 15.5 | 4.7 | 8.0 | |

**Behavioral Analyses.** The age of sexual maturity seems to differ depending on the species. Four-day-old *D. ercepeae* males already court conspecific females with maximal intensity, whereas *D. vallismaia* and *D. merina* males increase the vigor of their courtship between 7 and 11 d of age. Because of these marked ontogenetical variations, 8-d-old flies were chosen for the sexual isolation tests.

*Drosophila ercepeae* males initiated more courtship in front of *D. vallismaia* and mainly *D. merina* females than in front of their own females. Moreover, they copulated 3.5 times more often with *D. merina* females than with their conspecifics (Table 1). *D. merina* males slightly preferred courting conspecific females and only homospecific courtships led to copulations. *D. vallismaia* males discriminated clearly in favor of their own females, but also courted females of the other species a little; a few heterospecific copulations were obtained with *D. merina* females (Table 1).

*Drosophila merina* females copulated with all males although much less with those of *D. vallismaia*, whereas *D. ercepeae* females copulated only with conspecific males.

In conclusion, when pairs of flies were observed during 1 h—in the absence of food—heterospecific courtships were often observed, *D. merina* males being most promiscuous (33% courtships on average). However, homospecific copulations were always favored except for the case of *D. ercepeae* and *D. merina*, but depending on which sex is considered: *D. ercepeae* males with *D. merina* females led to more copulations, whereas *D. ercepeae* females as *D. merina* males prefer homospecific copulations (Table 1).

**Interspecific Hybridizations.** In the hybridization experiments, the conditions were different: larger groups (10 males and 10 females under no choice conditions) longer durations, and presence of food; thus, different results might be expected. Actually copulations, although few, were assessed between *D. vallismaia* males and *D. ercepeae* females and between *D. merina* males and *D. vallismaia* females, which courted only in the former conditions. These experiments allow study not only of the prezygotic but also of the postzygotic isolation barriers, as females were dissected to check for insemination.

Crosses between *D. ercepeae* and *D. merina* flies produced fertile male and female hybrids in both directions. This fertility is maintained during the following generations. Crosses between *D. vallismaia* males and *D. ercepeae* females or between *D. vallismaia* females and *D. merina* males produced a small number of sterile male and female hybrids. But crosses between *D. merina* females and *D. vallismaia* males did not produce any hybrids. Nevertheless, some sperm was visible inside the ventral receptacle and the spermathecae. Between *D. vallismaia* females and *D. ercepeae* males, no insemination could be detected (Table 2).

**Chemical Analyses of Cuticular Hydrocarbons.** Antony and Jallon (1982) and Jallon (1984) demonstrated that females of *D. melanogaster* and *D. simulans* possess cuticular hydrocarbons which act as contact pheromones, capable of inducing male courtship.

In the *ercepeae* complex, the composition of cuticular hydrocarbons of flies of each sex and each species involve up to 18 peaks, which correspond mainly to odd numbers of carbons (Table 3). They clearly show species-specific chemical signatures, but little sexual dimorphism (Fig. 30).

There are marked similarities, whatever the sex, between *D. ercepeae* and *D. merina* flies. All have more abundant cuticular hydrocarbons with 33 carbons (≈55.2%) and 31 carbons (≈29.2%) (Table 3). In both sexes of the 2 species, the main peaks

**Table 2. Interspecific hybridizations: all crosses were done under no choice conditions using 10 virgin males and 10 virgin females per tube**

| Females | Males | | |
|---|---|---|---|
| | *D. ercepeae* | *D. merina* | *D. vallismaia* |
| *D. ercepeae* | — | F1–F2 | Few F1 sterile |
| *D. merina* | F1–F2 | — | males and females |
| *D. vallismaia* | No hybrids | Few F1 sterile males and females | No hybrids — |

**Table 3. Percentages of the main cuticular hydrocarbons (mean ± SD) detected in 8-d-old *D. ercepeae*, *D. vallismaia*, and *D. merina* males and females (percentages represent relative peak areas among all peaks)**

| Carbon no. | *D. ercepeae* | | *D. vallismaia* | | *D. merina* | |
|---|---|---|---|---|---|---|
| | Male, $n = 4$ | Female, $n = 8$ | Male, $n = 10$ | Female, $n = 9$ | Male, $n = 4$ | Female, $n = 9$ |
| 22C[a] | 10.8 ± 9.7 | | 1.3 ± 1.1 | | 3.9 ± 1.6 | |
| 24C[a] | 43.1 ± 32.7 | | 40.1 ± 7.3 | | 83.3 ± 40.4 | |
| 25C | 5.4 ± 1.9 | 2.3 ± 0.8 | 2.0 ± 1.8 | 2.5 ± 1.3 | 5.0 ± 0.5 | 1.6 ± 0.7 |
| 28C | 4.2 ± 4.8 | 1.8 ± 1.6 | 0.1 ± 0.2 | 1.1 ± 1.4 | 4.1 ± 5.2 | 2.9 ± 2.8 |
| 29C | 1.0 ± 1.9 | | | 1.5 ± 1.6 | 1.4 ± 1.2 | |
| 30C | | | 0.3 ± 0.5 | 0.3 ± 0.4 | 1.4 ± 1.2 | |
| 31C 1 | | | 0.7 ± 0.6 | 1.5 ± 0.7 | | |
| 2 | 21.1 ± 2.2 | 23.3 ± 3.3 | 25.0 ± 2.2 | 27.3 ± 1.6 | 23.7 ± 4.1 | 21.9 ± 3.1 |
| 3 | 9.5 ± 6.1 | 7.3 ± 3.7 | 16.0 ± 2.4 | 19.9 ± 4.5 | | |
| 4 | 3.1 ± 2.8 | 1.7 ± 0.9 | 12.1 ± 1.4 | 12.2 ± 1.6 | | |
| 5 | 3.3 ± 2.3 | 3.3 ± 1.9 | 30.1[b] ± 3.7 | 28.0[b] ± 5.2 | | |
| 6 | | 1.1 ± 0.5 | 1.9 ± 1.0 | 0.8 ± 0.5 | | |
| 31C Total | 37.0 | 36.7 | 85.8 | 89.7 | 23.7 | 21.9 |
| 33C 1 | 1.1 ± 0.9 | 2.6 ± 2.9 | | | 0.4 ± 0.4 | 1.1 ± 0.2 |
| 2 | 5.4 ± 1.1 | 3.9 ± 0.7 | 0.7 ± 0.6 | | 4.4 ± 0.6 | 3.4 ± 0.6 |
| 3 | 15.1 ± 6.1 | 13.4 ± 2.7 | 3.5 ± 1.2 | 1.3 ± 0.6 | 18.5 ± 2.5 | 8.3 ± 2.6 |
| 4 | 9.2 ± 2.7 | 8.2 ± 1.4 | 1.8 ± 0.7 | 0.7 ± 0.7 | 8.50 ± 0.6 | 7.5 ± 0.6 |
| 5 | 21.6[b] ± 7.3 | 30.8[b] ± 4.1 | 5.7 ± 1.0 | 1.8 ± 0.9 | 27.5[b] ± 2.7 | 34.1[b] ± 3.1 |
| 33C Total | 52.4 | 58.9 | 11.7 | 3.8 | 59.3 | 54.4 |
| 35C 1 | | | | | 5.3 ± 1.5 | 11.5 ± 3.1 |
| 2 | | | | | | 1.7 ± 1.5 |
| 3 | | | | | 0.3 ± 0.5 | 5.5 ± 2.1 |
| 35C Total | | | | | 5.6 | 18.7 |

[a] Noncuticular hydrocarbons.
[b] Major compounds.

comigrate in GC, 33C5, a diene, and 31C2, an alkane. The former is larger in females than in males. The latter peak is also abundant in both sexes of *D. vallismaia* (≈26.2%) but, in that species, is smaller than another peak corresponding to a 31C diene, 31C5, which is weak or nonexistent in the other species.

There are also differences between *D. ercepeae* and *D. merina*: cuticular hydrocarbons with 35C are not detectable in the former species but they are present in *D. merina*, especially in females. Moreover, peaks 31C3-5 are absent in the latter species; peak 33C3 is also smaller in *D. merina* females (Table 3).

In *D. vallismaia*, cuticular hydrocarbons with 31C are the major compounds in each sex (≈88%); 31C2, an alkane, is one of the main peaks, as in the other species, and 31C3-5 correspond to trienes and dienes, according to their mass spectra (not shown).

Measurements of cuticular hydrocarbons total amounts point out new differences. No quantitative differences between males and females in *D. vallismaia* and *D. merina* (1,637 ng per fly on average), but a factor of 2 between both sexes of *D. ercepeae*, more cuticular hydrocarbons in females (Table 4).

There is also an abundant male-specific compound in all species which is not a hydrocarbon. This compound might be a long chain acetate homologous to vaccenyl acetate, which is also present, although in smaller amounts, as in the males of the *D. ananassae* complex (Schaner et al. 1989).

The chemical picture described here is characterized by a limited sexual dimorphism; it recalls the situation in *D. simulans* and 4 other species of the *melanogaster* subgroup (Jallon 1984, Jallon and David 1987). In these species, a common cuticular hydrocarbon, 7-tricosene, although abundant in both sexes, plays an important role in triggering male precopulatory behavior. Male *D. vallismaia* courted more their own females, but a few copulations were obtained with *D. merina* females in pairs during short times—in the absence of food—and a few hybrids were obtained with *D. ercepeae* females. An abundant unsaturated cuticular hydrocarbon with 31C, present in their cuticle, rare in the cuticles of other species, might play an important behavioral role in *D. vallismaia*. Also, in short experiments using couples, *D. ercepeae* males courted the 3 types of females, and *D. merina* females were preferred; whereas, *D. merina* males courted the 3 types of females equally. In short durations, only homospecific copulations were obtained, but in long durations with food, some progeny was obtained with *D. vallismaia* females.

Cuticles of *D. ercepeae* and *D. merina* females have abundant unsaturated 33C compounds, whereas *D. vallismaia* females have unsaturated compounds with 31C. Males of *D. ercepeae* and *D. merina* might be reacting to such compounds, especially 33C5. However, it is quite possible that male compounds also play roles toward females. Also, other sensory cues have to be taken into account.

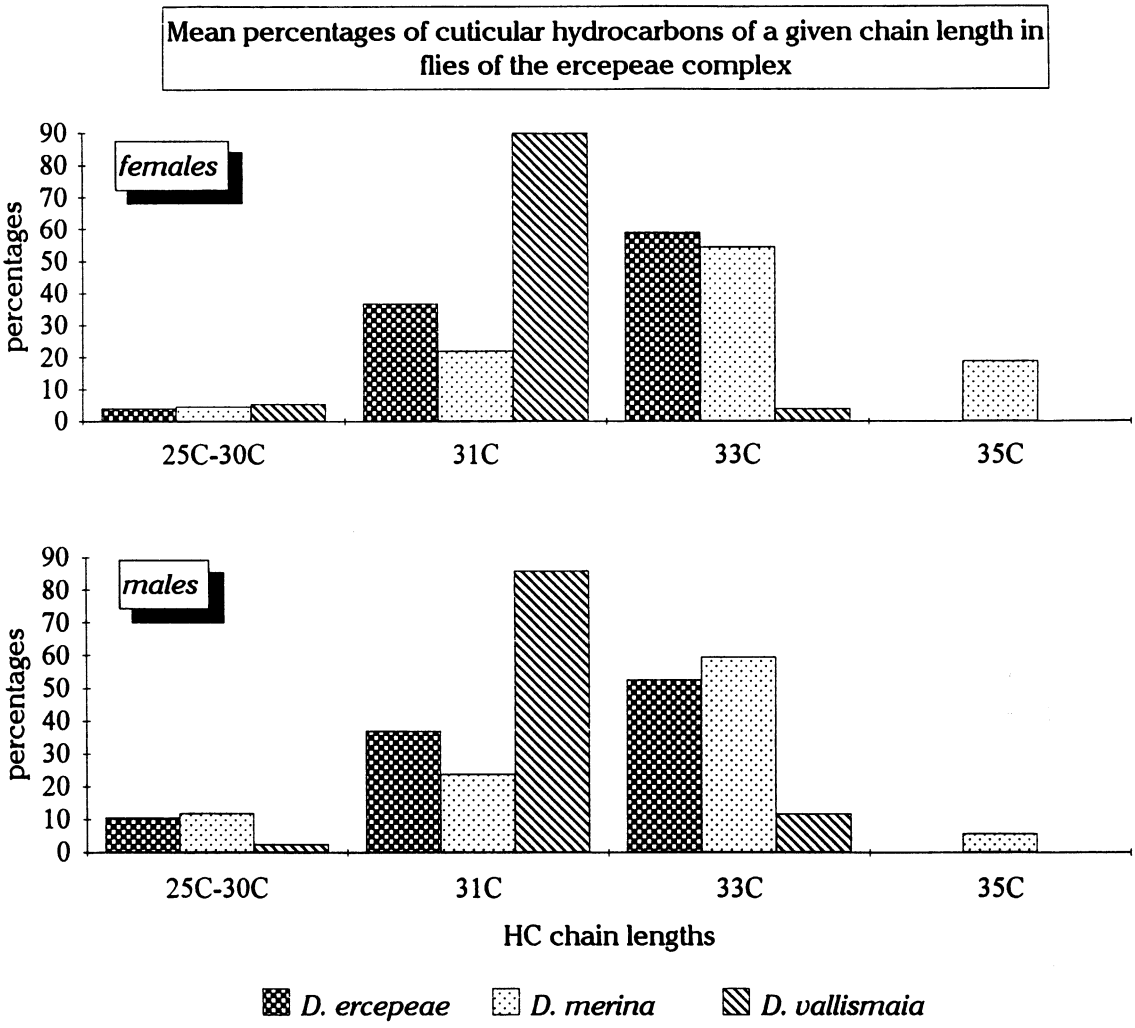



**Fig. 30.** Mean percentages of cuticular hydrocarbons of a given chain length in females and males of the *ercepeae* complex.

Until now, female sex pheromones have been identified by chemical means in only 3 species of *Drosophila, D. melanogaster, D. simulans,* and *D. virilis*: (Z,Z),7,11-heptacosadiene, (Z)-7-tricosene, and (Z)-11-pentacosene, respectively (Jallon 1984, Antony et al. 1985, Oguma et al. 1992). An aggregation pheromone of *D. ananassae* has been identified, (Z)-11-octadecenyl acetate, which is also found in the *D. melanogaster* species subgroup (Schaner et al. 1989).

**Amylase Electrophoretic Patterns.** Amylase is one of the more extensively enzymatic systems studied for both protein polymorphism and DNA variation in a number of *Drosophila* species. Kikkawa (1964) and Doane (1969) described several electrophoretic variants of amylase in *D. melanogaster.* Daïnou et al. (1987) reported 13 different electromorphs in this species, which was considered to be the most polymorphic for amylase until *D. ananassae* was studied. However, the 1st results in *D. ananassae* adults (Kikkawa 1964, Doane 1969) revealed a limited polymorphism, a few individuals having only 2 or 3 bands. The complexity of the amylase system in *D. ananassae* was discov-

**Table 4. Average total amounts of cuticular hydrocarbons (mean ± SD)**

| *D. ercepeae* | | *D. vallismaia* | | *D. merina* | |
|---|---|---|---|---|---|
| Female | Male | Female | Male | Female | Male |
| 2,185.3 ± 673.5 | 1,095.4 ± 166.7 | 1,652.5 ± 390.9 | 1,722.4 ± 690.8 | 1,587.8 ± 294.0 | 1,587.0 ± 707.6 |

ered only by analyzing African collections of this species. Da Lage et al. (1989) surveyed the geographical distribution of amylase extensively. Altogether, 13 electromorphs, numbered according to their electrophoretic mobility, have been recorded (Cariou and Da Lage 1993). Thus, *D. ananassae* appears at least as polymorphic as *D. melanogaster.* The amylase pattern in the *ercepeae* complex was analyzed by Da Lage et al. (1989). This pattern is quite different from the one found in *D. ananassae* and presents unique isoamylase variants. Instead of the polymorphic pattern described for *D. ananassae, D. ercepeae, D. vallismaia,* and *D. merina* share a unique band, which is slightly slower than Amy 4 of *D. ananassae* (Amy 4a). *D. merina* shows a 2nd faster isoamylase (Amy -1a). This simpler pattern is not restricted to the *ercepeae* complex. In their survey, Da Lage et al. (1989) studied several other species of the *ananassae* subgroup: 4 species of the *bipectinata* complex (*D. malerkotliana, D. bipectinata, D. parabipectinata,* and *D. pseudoananassae*), *D. monieri* belonging to the *ananassae* complex, and *D. varians* (uncertain position). In all these species, single-banded phenotypes predominate. The species of the *bipectinata* complex have a common band, slightly faster than Amy 2 of *D. ananassae.* This band may correspond to the same allele in the 4 species. In addition, apart from *D. bipectinata,* some individuals of the other species show a 2nd common band, faster than the former. *D. monieri* and *D. varians* have a single amylase band which has the same mobility as the *D. ananassae* isoamylase 3. All the other species of the *ananassae* subgroup studied by Da Lage et al. (1989) have a weaker amylase activity and a more reduced polymorphism than does *D. ananassae.* This appears similar to what is observed within the *melanogaster* species subgroup (Daïnou et al. 1987), where all the species have much less amylase variation than *D. melanogaster.*

## Discussion

The relationships within the *ercepeae* complex are summarized in Fig. 31. On the basis of the morphological data—the only data available for *D. comorensis*—we may distinguish *D. comorensis* and *D. vallismaia* from *D. ercepeae* and *D. merina.* The other characters all demonstrate the close affinities between *D. ercepeae* and *D. merina.* Under laboratory conditions, these 2 species can produce fertile hybrids whichever the direction of the crosses, whereas those involving *D. vallismaia* either fail to give any progeny or yield only a few sterile male and female hybrids. However, data on Hawaiian Drosophilidae (Kaneshiro 1980, Kaneshiro and Giddings 1987, Carson and Yoon 1982) indicate that hybrid fertility is not necessarily an indication of the degree of relationship between species pairs. *D. ercepeae* and *D. merina* share more abundant cuticular hydrocarbons with 33 carbons. In both sexes of both species, the main compound is a diene 33C5, which may be a common contact sex pheromone. This compound is found rarely in *D. vallismaia* females, where the most abundant cuticular compound is a diene with 31 carbons. The fact that *D. ercepeae* and *D. merina* court *D. vallismaia* females willingly may suggest close structural relations between these dienes with 31 and 33 carbons; but *D. vallismaia* males are more selective. However, *D. merina* have extra hydrocarbons with 35 carbons and a private amylase isoform (Amy -1a), both characters not found in *D. vallismaia* or *D. ercepeae.* These observations suggest that *D. merina* evolved more rapidly than *D. ercepeae* and *D. vallismaia.*The cytogenetic data are also consistant with the closeness of *D. ercepeae* and *D. merina.* Although the 3 species have homosequential polytene chromosomes, mitotic chromosomes of *D. vallismaia* differ from the other 2 by the shape and the structure of the 4th metacentric pair.

It is generally considered that the *melanogaster* species group originated in Southeast Asia, where 60% of all species and 8 of the 12 subgroups are present. From there, the group has dispersed and shows secondary radiations in nearby biogeographical regions (Australian, African, and east Palearctic). The presence in each of these regions of endemic subgroups indicates that these dispersals were ancient. It is generally considered that the *ananassae* subgroup also arose in Southeast Asia, where 9 species are endemic.

The distribution of the *ananassae* subgroup has already been discussed by several authors (Bock 1980, Tsacas 1984, Lemeunier et al. 1986, McEvey et al. 1987). More recently, Tobari (1993) describes the distributions of 10 of 11 species of the *ananassae* complex. Before discussing the comparative distribution of the species of the 3 complexes of the *ananassae* subgroup, it is necessary to include some preliminary comments. First, we shall not include the distribution of *D. ananassae* and *D. malerkotliana,* which are very widespread and subcosmopolitan. Second, we shall also not consider the 3 species *D. adamanensis, D. micropectinata,* and *D. pereirae,* because each of them was described from only one place; moreover, they do not fit into any of the 3 complexes and some authors even disagree about their position within the *ananassae* subgroup (Takada et al. 1973, Takada and Momma 1975, Bock 1980).

The *ananassae* subgroup occupies a large area from occidental Africa (west) to Touamutu Islands (east). This area may be divided into 5 zones from west to east as follow (Fig. 32):

Zone A, the most occidental area, includes the African continent, in which *D. lachaisei,* an endemic species, is the only species belonging to the *ananassae* complex. The presence of *D. lachaisei* in West Africa, far from any other species of the complex, is difficult to explain. One could imagine that the introduction of *D. lachaisei* or of its ancestor in occidental Africa happened during an an-

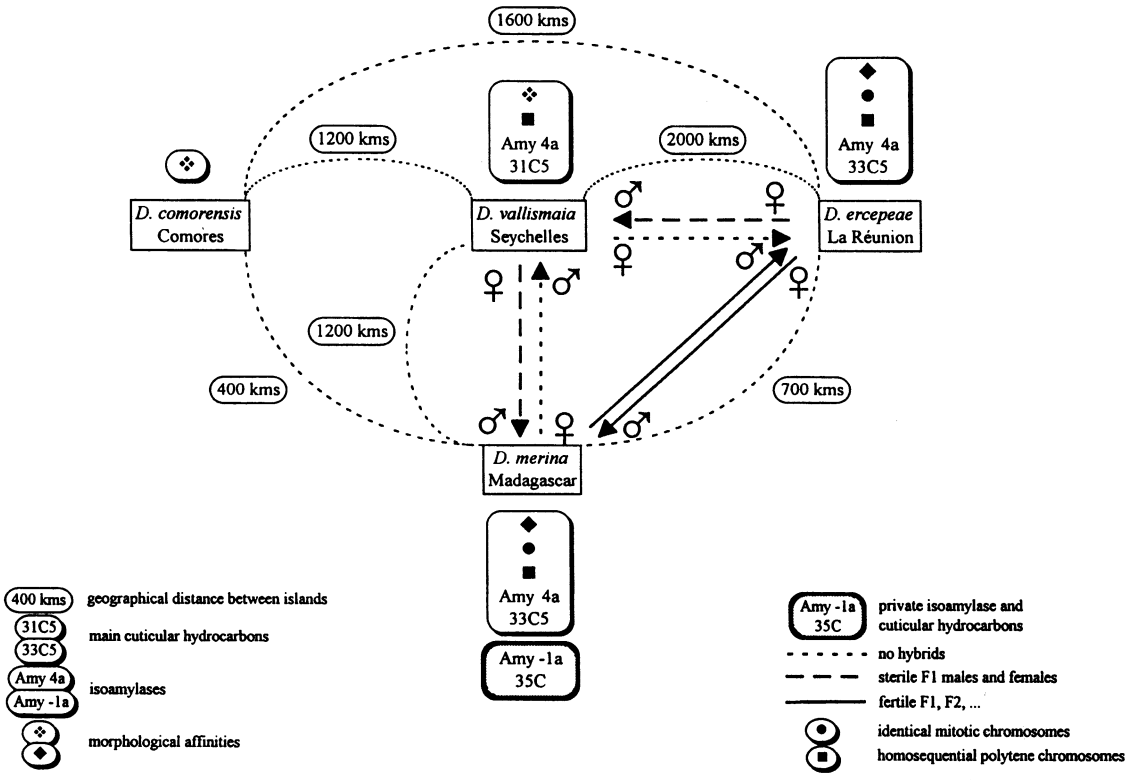



**Fig. 31.** Schematic representation of the relationships between the 4 species of the *ercepeae* complex.

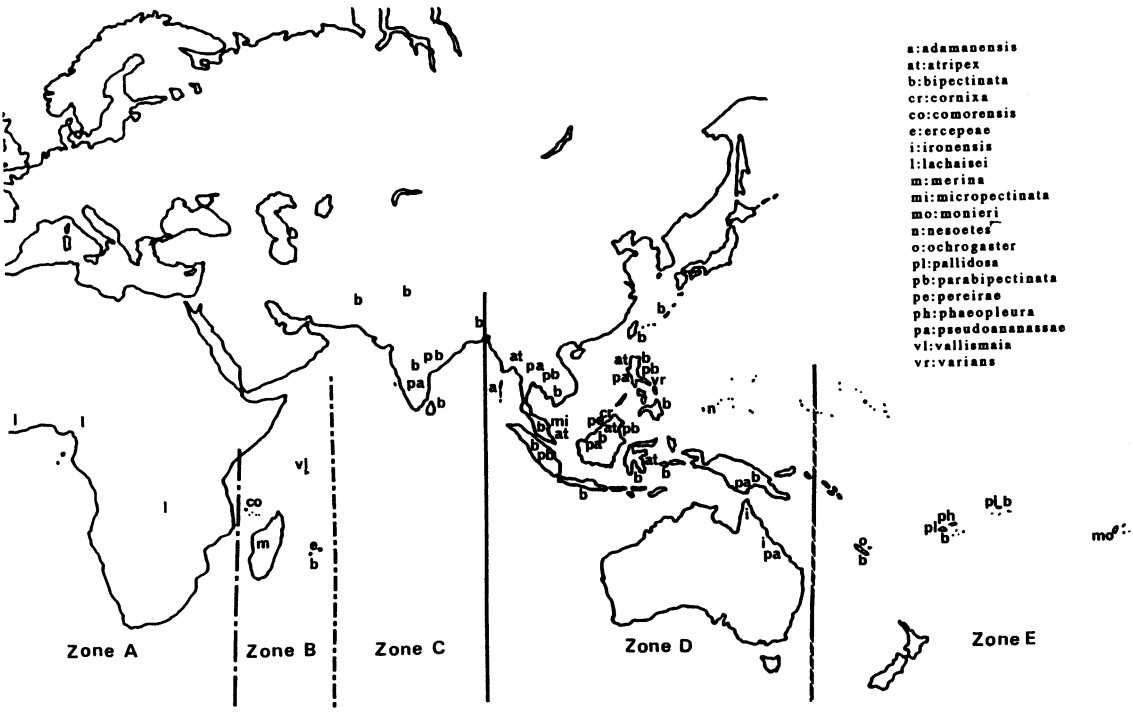



**Fig. 32.** Geographic distribution of the *Drosophila ananassae* subgroup species (the cosmopolitans *D. ananassae* and *D. malerkotliana* are not included).

cient time by terrestrial introduction. Because of climatic changes that gave rise to the desertification of most of the area between northern India and Africa, the ancestor of *D. lachaisei* might have been geographically isolated.

Zone B includes the islands of the occidental part of the Indian Ocean, including Madagascar. The 4 endemic species of the *ercepeae* complex are present together with one species belonging to the widely distributed *bipectinata* complex (*D. bipectinata*). The *ananassae* complex is absent.

Zone C includes the Indian subcontinent, Sri Lanka, and the Adaman and Nicobar islands. Only 2 widespread species (*D. bipectinata* and *D. pseudoananassae*) belonging to the *bipectinata* complex occur here. The *ananassae* complex is absent, which is surprising because this wide area is close to the main geographical range of the *ananassae* complex species.

Zone D comprises Southeast Asia, Malaysia, Indonesia, the Philippines, New Guinea, and Australia. In this zone, 5 endemic species of the *ananassae* complex are present (*D. atripex, D. cornixa, D. ironensis, D. nesoetes,* and *D. varians*) with 3 nonendemic species of the *bipectinata* complex (*D. bipectinata, D. parabipectinata,* and *D. pseudoananassae*). The great species diversity of this area might be explained by the existence of numerous islands that probably favored the radiation of the *ananassae* subgroup. Zone D occupies the center of the distribution area of the subgroup. This area is also considered the origin area of the *melanogaster* group (Bock and Wheeler 1972) and maybe, even, of the Drosophilidae itself (Throckmorton 1975).

Zone E corresponds to Oceania east of New Guinea and Australia. Four species of the *ananassae* complex (*D. monieri, D. ochrogaster, D. pallidosa,* and *D. phaeopleura*) are found with 1 nonendemic species of the *bipectinata* complex (*D. bipectinata*). According to Tobari's map, *D. atripex* is also present, in New Caledonia. Tobari (personal communication) confirmed that she drew *atripex*'s distribution using McEvey et al. (1987) and Tsacas and Chassagnard (1988) papers, but further studies of the supposed *D. atripex* strain from Noumea allowed Chassagnard and Groseille (1992) to conclude that it was not *D. atripex* but a new species, *D. ochrogaster* Chassagnard.

Zone B is the only one where the *ercepeae* complex is found. The 4 species of this complex thus probably differentiated here, by the fortuitous introduction of an ancestor whose origin remains obscure. This origin might have been African or Oriental. The 1st hypothesis requires that the African continent was already colonized by one or several species of the *ananassae* subgroup, either totally or, at least, in its eastern part. Such a colonization would be supported by the presence, although only in West Africa, of *D. lachaisei*. The 2nd hypothesis seems more probable for the following reasons. The migration of *Drosophila* from the Oriental region to the occidental Indian Ocean islands seems possible (Bock and Wheeler 1972, Tsacas 1984). As already shown, the species of the *ercepeae* complex are closer to the species of the *bipectinata* complex (*D. bipectinata, D. parabipectinata,* and *D. pseudoananassae*). This complex is found in the part of the Oriental region closer to the occidental Indian Ocean islands where *D. bipectinata* is actually sympatric with the species of the *ercepeae* complex. One may imagine that the *ercepeae* complex would have radiated from an ancestral species that already possessed "a bifid phallus without an individualized distiphallus," or some similar structure. This ancestral species might have been introduced in the islands of the occidental Indian Ocean long enough to allow the radiation of the *ercepeae* complex.

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