Between species divergence of cyst length distributions in the Drosophila melanogaster species complex

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ABSTRACT

The cyst lengths in each of the four cryptic species of the Drosophila melanogaster complex are stable. There is no variability due to the geographic origin of lines or strains. There is strong evidence that the distribution of cyst length is a highly species-specific trait. The extent to which the cyst length distributions of the four species diverge, is striking; two species (D. mauritiana and D. simulans) have short cysts while the others (D. sechellia and D. melanogaster) have long cysts. There is partial overlap within but not between these long and short distributions. In D. mauritiana and D. simulans where the overlap is the greatest the variability is the least. Although D. mauritiana, D. simulans and D. sechellia make a monophyletic group with respect to D. melanogaster, D. sechellia appears to have strongly diverged from the mauritiana-simulans pair. The cyst length of an hypothetical common ancestor can not be inferred from these studies.

1. INTRODUCTION

In the Drosophila genus sperm length varies between species by about 1400 fold (from 0.010 mm in D. azteca to 14 mm in D. hydei) (Yanders and Perras 1960, 1963; Hess and Meyer 1963; Beatty and Burgoyne 1971; Hatsumi and Wakahama 1986; Joly, unpubl.). The question arises: why is there so extensive a variation in so limited a taxon? The purpose of this study is to examine whether sperm length shows a species-specific stability in a complex of closely related species such as the melanogaster complex or whether there is a sperm length polymorphism. Hence, two further questions can be raised: i) Is there a polymorphism between-isofemale lines? In species like D. melanogaster where multiple mating accompanied by sperm predominance may occur (Gromko and Pyle 1978; Gromko et al. 1984) and where females possess a mechanism for selecting specific sperm and therefore exhibiting sperm preference (Childress and Hartl 1972), this may have resulted in strong sperm competition (sensu Parker 1970). In the D. obscura group where several sperm size classes exist within one male gonad (Policansky 1970; Beatty and Sidhu 1970; Beatty and Burgoyne 1971; Kurokawa et al. 1974; Joly et al. in prep.) the distribution of the size classes has been assumed to reflect specializations for competition at different times of the female’s reproductive
life (Sivinsky 1980). ii) Is there variability between-geographic strains? Do the two cosmopolitan species—D. melanogaster and D. simulans—which have widespread, albeit scattered, large populations vary in sperm length? It is shown here that the four sibling species of the melanogaster complex differ in sperm length and that this has a species-specific distribution because there is no noticeable intraspecific variation. Drosophila mauritiana and D. simulans are phenetically close while strongly differing from D. sechellia which lies nearer to D. melanogaster.

2. MATERIALS AND METHODS

For between-isofemale line comparison sperm length analysis was carried out for each of the three closely related species of the simulans lineage by examining four isofemale lines, each issued from one wild-caught inseminated female. These were: Drosophila sechellia, Seychelles, from Cousin Is.: lines 2, 15 and 25; and from Frigate Is.: line 1; 1985 (Gif 267-2). D. mauritiana, Mauritius, from Les Galets: lines LG67, LG72, LG74 and LG76; 1985 (Gif 275-1). D. simulans, Seychelles, from Mahé Is.: lines 9, 11, 13 and 21; 1981 (Gif 229-3).

For between-strain comparison in the two cosmopolitan species strains found by more than one wild-caught inseminated female were used. In D. melanogaster three strains were chosen from different geographic origins: Ivory Coast, Tai, 1983 (Gif 255.1); South Africa, Cape Town, 1984 (Gif 263.2); France, Yquem, 1986 (Gif 272.1).

In D. simulans four strains were chosen on the basis of their different mitochondrial DNA patterns as recognized in Baba-Aissa and Solignac (1984) and Solignac and Monnerot (1986): Si 1: “Indo-Pacific race” from Mahé Is., Seychelles, 1981 (Gif 229.2); Si 2: “Cosmopolitan race” from Draveil, France, 1986 (Gif 273.1) and from Brazzaville, Congo, 1981 (Gif 246.1); Si 3: “Malagasy race” from Mt Ambre, Madagascar, 1980 (Gif 248.1).

The cultures were reared at 23°C. Virgin males and females were aged for 5 days and then placed together. The testes were dissected out in a drop of ringer solution on a microscope slide. The testis was held apically and ruptured basally, at the contact with the seminal vesicle, by thin steel needles. It was then moved to, and then around, the border of the drop and shaken gently in order to provoke the removal of the content of the sperm bundle. Measurement of mature cysts was preferred to that of spermatozoa since it avoids scoring broken sperm and therefore provides more reliable data. A mature cyst was defined as being one in which sperm heads are in the process of emerging like a brush from the apical open end of the cyst. A median measurement is made of the sperm bundle from the top of the sperm
heads to the bottom of the waste bag, the final form of the cystic bulge at its caudal end (Tokuyasu et al. 1972). Cysts were traced with the aid of a camera lucida and the trace lengths measured on a digitizing table connected to a micro-computer. Fifty cysts of more than five testes were prepared for each strain.

3. RESULTS

The cyst lengths are summarized in three figures showing consecutively the between-isofemale line variability in *D. mauritiana*, *D. simulans* and *D.

Fig. 1. Between-isofemale line variability of cyst length distribution in the three closely related species of the *Drosophila simulans* lineage. *D. mauritiana* (from top to the bottom: lines 67, 74, 76 and 72); *D. simulans* (lines 21, 13, 11 and 19); *D. sechellia* (lines 2, Frigate, 25, 15).
sechellia (Fig. 1), the between-geographic strain variability in the two cosmopolitan species *D. simulans* and *D. melanogaster* (Fig. 2) and the four species of the *melanogaster* species complex (Fig. 3).

Fig. 1 and 2 show that cyst length distribution is stable regardless of
whether the scores are made of lines of the same, or of different, geographic origin. As a result, interspecific comparisons can be made between any sets of data. This consistency reinforces the reliability of the measure. Thus there is strong evidence that cyst length distribution is a highly species-specific trait.

Fig. 3 combines all the data provided by both lines and strains for the four melanogaster complex species. Two major points can be emphasized: i) the magnitude of the divergence of the cyst length distributions of the four cryptic species is very large, ii) two categories can be recognized with either short cyst species (D. mauritiana and D. simulans) or long cyst species (D. sechellia and D. melanogaster). Compared to the within category overlap there is almost no between category overlap. However, the extent of the overlaps varies from one phenotypic species pair to another being large between D. mauritiana and D. simulans while limited between D. sechellia and D. melanogaster. It is worth noting that the narrow distribution of D. mauritiana and D. simulans is constant while that of D. sechellia varies slightly between lines. The extent of that variation is nearly the same as the difference between D. mauritiana and D. simulans.

4. DISCUSSION

The four species of the melanogaster complex are closely related to one another. There is now good evidence that Drosophila mauritiana, D. simulans and D. sechellia are more closely related to one another than either is to D. melanogaster (see review in Lemeunier and Ashburner 1984; Coyne and Kreitman 1986; Lachaise et al. 1987 and references therein).

However, there are very few morphological traits that can be used for distinguishing them. These include the shape of the posterior process of the genital arch in males, which is a diagnostic character (Lemeunier et al. 1986) and sex comb teeth which is a less reliable trait for identification due to noticeable variability (Bock and Wheeler 1972; Coyne and Kreitman 1986; Tsacas 1971; Tsacas and David 1974, 1978; Tsacas and Lachaise 1974). Note also that the color of the testes can be used for separating D. mauritiana from the other species (Coyne and Kreitman 1986).

A similarity analysis of cyst length was made showing their distributions to be species-specific despite partial overlap. Hence, considering the limited set of morphological traits available for allowing identification, cyst length distribution appears as a novel and useful tool. Sperm length was shown formerly to be species-specific as for example in the Drosophila nasuta sub-group by Hatsumi and Wakahama (1986).

The question about possible relationship between cyst length similarity
and systematic relatedness comes across the usual snag of assuming a phy-
logeny from a single character. This is made clear by the various single in-
dependent characters mentioned above producing inconsistent phylogenies. 
Since *D. mauritiana*, *D. simulans* and *D. sechellia* unequivocally make a 
monophyletic group with respect to *D. melanogaster*, the similarity between 
the cyst length distributions of *D. sechellia* and *D. melanogaster*, which both 
have long cysts, may be due to parallel evolution. As a result, *D. sechellia* 
and the *mauritiana-simulans* pair, appear to have strongly diverged. The 
question is whether both or only one of them diverged from the cyst length 
pattern of their common ancestor?

Kurokawa and Hihara (1976) suggested a relation between the number of 
primary spermatocytes per cyst and the phylogeny, with a reduction in more 
advanced forms accompanied by an increase in the size of sperm. But they 
found a similar number of primary spermatocytes per cyst for *D. melano-
gaster* and *D. simulans* that leads to the expectation that they have a similar 
sperm length. However, it is shown here that the cyst length distributions 
of these two species discriminate.

Any attempt at assuming which cyst length pattern is ancestral and which 
derived would require knowledge of the pattern of their common ancestor. 
Outgroup analysis could only be useful in this respect provided there has not 
been extensive parallel or convergent evolution for that character. It is 
doubtful that there is an evolutionary trend towards increase through time. 
Rather the striking variability of cyst lengths found in these four closely 
related species suggests that such morphological change occurs relatively 
quickly during the speciation process.

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