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 founded by more than one wild-caught inseminated female were used. In *D. melanogaster* three strains were chosen from different geographic origins: Ivory Coast, Taï, 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 ofringer 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

Fig. 2. Between-geographic strain variability of cyst length distribution in the two cosmopolitan species *D. simulans* (from top to bottom: Draveil, France; Ambre, Madagascar; Brazzaville, Congo); *D. melanogaster* (Yquem, France; Cape Town, South Africa; Tai, Ivory Coast).

Fig. 3. Species-specificity of the cyst length in the four species of the *Drosophila melanogaster* complex (maur: *D. mauritiana*; sim: *D. simulans*; sech: *D. sechellia*; mel: *D. melanogaster*).

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* subgroup 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 phylogeny from a single character. This is made clear by the various single independent 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. melanogaster* 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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REFERENCES

- BABA-AISSA, F. and SOLIGNAC, M. (1984) La plupart des populations de *Drosophila simulans* ont probablement pour ancêtre une femelle unique dans un passé récent. *C. R. Acad. Sci. Paris* **299**, Ser III, 289-292.
- BEATTY, R. A. and BURGOYNE, P. S. (1971) Size classes of the head and flagellum of *Drosophila* spermatozoa. *Cyto genetics* **10**, 177-189.
- BEATTY, R. A. and SIDHU N. S. (1970) Polymegaly of spermatozoan length and its genetic control in *Drosophila* species. *Proc. Roy. Soc. Edinburgh* (B) **71**, 14-28.
- BOCK, I. R. and WHEELER, M. R. (1972) The *Drosophila melanogaster* species group. *Univ. Texas Publ.* **7213**, 1-102.
- CHILDRESS, D. and HARTL, D. L. (1972) Sperm preference in *Drosophila melanogaster*. *Genetics* **71**, 417-427.
- COYNE, J. A. and KREITMAN, M. (1986) Evolutionary genetics of two sibling species *Drosophila simulans* and *D. sechellia*. *Evolution* **40**, 673-691.
- GROMKO, M. H. and PYLE, D. W. (1978) Sperm competition, male fitness, and repeated mating

- by female *Drosophila melanogaster*. *Evolution* **32**, 588-593.
- GROMKO, M. H., GILBERT, D. G. and RICHMOND, R. C. (1984) Sperm transfer and use in the multiple mating system of *Drosophila*. In "*Sperm competition and the Evolution of Animal Mating Systems*" (ed. R. L. Smith). pp. 371-426. Academic Press, New York.
- HATSUMI, M. and WAKAHAMA, K. I. (1986) The sperm length and the testis length in *Drosophila nasuta* subgroup. *Jpn. J. Genet.* **61**, 241-244.
- HESS, O. and MEYER, F. (1963) Chromosomal differentiations of the lampbrush type formed by the Y chromosome in *Drosophila hydei* and *D. neohydei*. *J. Cell. Biol.* **16**, 527-539.
- KUROKAWA, H. and HIHARA, F. (1976) Number of first spermatocytes in relation to phylogeny of *Drosophila* (Diptera: Drosophilidae). *Int. J. Insect Morphol. and Embryol.* **5**, 51-63.
- KUROKAWA, H., MATSUO, Y. and HIHARA, F. (1974) A study on sperm length and body size of *Drosophila bifasciata*. *Annotations Zoologicae japonenses* **47**, 140-146.
- LACHAISE, D., CARIQU, M.-L., DAVID, J. R., LEMEUNIER, F., TSACAS, L. and ASHBURNER, M. (1987) Historical biogeography of the *Drosophila melanogaster* species subgroup. *Evolutionary Biology* **22**. In press.
- LEMEUNIER, F. and ASHBURNER, M. (1984) Relationships within the *melanogaster* species subgroup of the genus *Drosophila* (Sophophora). IV. The chromosomes of two new species. *Chromosoma* **89**, 343-351.
- LEMEUNIER, F., TSACAS, L., DAVID, J. R. and ASHBURNER, M. (1986) The *melanogaster* species group. In "*The genetics and Biology of Drosophila*" Vol. 3e. (eds. M. Ashburner, J. N. Thompson, Jr. and H. L. Carson). pp. 147-256. Academic Press, London.
- PARKER, G. A. (1970) Sperm competition and its evolutionary consequences in the insects. *Biol. Rev. Com. Philos. Soc.* **45**, 525-567.
- POLICANSKY, D. (1970) Three sperm sizes in *D. pseudoobscura* and *D. persimilis*. *Dros. Inf. Serv.* **45**, 119-120.
- SIVINSKY, J. (1980) Sexual selection and insect sperm. *Florida Entomologist* **63**, 99-109.
- SOLIGNAC, M. and MONNEROT, M. (1986) Race formation, speciation, and introgression within *Drosophila simulans*, *D. mauritiana*, and *D. sechellia* inferred from mitochondrial DNA analysis. *Evolution* **40**, 531-539.
- TOKUYASU, K. T., PEACOCK, W. J. and HARDY, R. W. (1972) Dynamics of spermiogenesis in *Drosophila melanogaster*. II. Coiling process. *Z. Zellforsch.* **127**, 492-525.
- TSACAS, L. (1971) *Drosophila teissieri*, nouvelle espèce africaine du groupe *melanogaster* et note sur deux autres espèces nouvelles pour l'Afrique. *Bull. Soc. Ent. France* **76**, 35-45.
- TSACAS, L. and DAVID, J. R. (1974) *Drosophila mauritiana*, n. sp. du groupe *melanogaster* de l'île Maurice (Diptera, Drosophilidae). *Bull. Soc. Ent. Fr.* **79**, 42-46.
- TSACAS, L. and DAVID J. R. (1978) Une septième espèce appartenant au sous-groupe *Drosophila melanogaster* Meigen: *Drosophila orena* spec. nov. du Cameroun (Diptera: Drosophilidae). *Beitr. Ent. Berlin* **28**, 179-182.
- TSACAS, L. and LACHAISE, D. (1974) Quatre nouvelles espèces de la Côte-d'Ivoire du genre *Drosophila*, groupe *melanogaster*, et discussion de l'origine du sous-groupe *melanogaster* (Diptera: Drosophilidae). *Ann. Univ. Abidjan, E*, **7**, 193-211.
- YANDERS, A. F. and PERRAS, J. P. (1960) Sperm length in four *Drosophila* species. *Dros. Inf. Serv.* **34**, 112. Corrected 1963, *Dros. Inf. Serv.* **38**, 145.