Lachaise P., J Lemeunier & M. Veuille, 1981

CLINAL VARIATIONS IN MALE GENITALIA IN *DROSOPHILA TEISSIERI* TSACAS

Male genitalia are a well-known means for distinguishing species in insects. Their structural stability within each species permits systematic scientists to commonly use them to identify species.

Snodgrass (1957 p. 1) wrote, "The great diversity in structural detail of the genitalia gives these organs a value for the identification of insect species almost equal to that of fingerprints for the identification of human individuals." The striking association of each species with a given type of genitalia formerly led scientists to believe that the reproductive apparatus had an active role to play in the process of speciation. Reproductive isolation is universally recognized as a major step in speciation, but it does not seem to require any morphological changes in most cases (Wiley 1978). A review by Dobzhansky (1951) of the experimental studies so far carried out about the genitalia of insects led him to assume that there was no evidence that mechanical isolation was actually used as an effective barrier against hybridization. According to Mayr (1969) the structural changes in genitalia are a consequence of speciation rather than a possible cause. They appear as a possible by-product of the overall genetical changes associated with speciation. Nevertheless, the dogma of species recognition based upon differentiation of genitalia is still used nowadays, since genitalia remain the main characters for investigating the diversity in insect faunas.

Therefore, the situation described here for *Drosophila teissieri* is somewhat different since the geographical differentiation of male genitalia allows the discrimination between strains from various origins with a probability of near certainty of visual recognition of the furthermost populations. Subspecific morphological changes are infrequent in drosophilids. It was tempting to correlate this external divergence with some incipient genetical differentiation.

The available strains would not have yielded good estimates for allozyme frequencies in the wild, but they were convenient for a comparison of chromosomal variation and for the study of pre- and postmating isolation. The strains tested originated from several locations spread throughout the range of this species in tropical Africa.

MATERIALS AND METHODS

Laboratory strains.—*Drosophila teissieri* is a member of the Afrotropical *melanogaster* subgroup. Widespread from the Sahel to southern Africa, this species is more especially abundant in highland areas. The strains used in this study originated from different localities as follows: 1) Nimba mountains (boundary of Guinea and Ivory Coast). Eight strains were collected from different altitudes between 650 and 1300 m; 2) Lamto (Ivory Coast); 3) Bafut N'guemba (Cameroon); 4) N'Kolbisson (near Yaoundé, Cameroon); 5) Ozon (near Yaoundé, Cameroon); 6) Zoatoupsi (near Yaoundé, Cameroon); 7) Makokou (Gabon);

8) Brazzaville (Congo), two strains; 9) Selinda mountains (near Chipinda, Rhodesia). In addition to these strains, a sample of preserved specimens from Bunyakiri on the Luhoho river (near Lake Edwards, Zaïre) was examined for morphological analysis. The two strains from Brazzaville were collected 3 yr apart (1974 and 1977).

Morphological analysis.—Although they are included in the description of genitalia by taxonomists, the anal plates of drosophilids belong to a metamere posterior to those bearing the proper genitalia structures (Hollingsworth 1960; Tsacas et al. 1971). They are coadapted to the genital arch and contribute to the make-up of the functional male apparatus.

In *Drosophila teissieri*, the anal plates bear characteristic thick teeth which have a diagnostic value. A scanning electron microscope study of fixed mated individuals revealed these teeth gripping the female ovipositor, suggesting that they were involved in the insemination process.

Cytological analysis.—Temporary squash preparations were made following the techniques described by Ashburner (1967).

Reproductive isolation tests.—Four strains of *Drosophila teissieri* were examined in isolation tests (Nimba, Lamto, N'Kolbisson, and Selinda). Postmating hybridization tests used pairs composed of a virgin female and a newly emerged male. F_1 crosses (243 assays) and each kind of backcross (416 assays) were performed.

Tests of premating preferences between pairs of strains were adapted from the multiple choice method of Ehrman and Petit (1968). Ten males and 10 females from each strain were kept in isolated tubes for 24 h after emergence. Males and females from one strain had the edge of the wing slightly clipped to allow recognition. The marked strains were alternated in parallel runs. In order to avoid the stress due to etherization before marking, the flies were anesthetized by transfer into tubes cooled on ice. The virgin flies were aged up to 5 days and then introduced into mating chambers. These were adapted from Elens and Wattiaux (1964) and Ehrman (1965, 1968). They were made from plastic petri dishes whose covers had been replaced by a fine gauze.

The 40 individuals were introduced simultaneously through an opening on the side of the chambers. Preliminary runs showed that the time required for half the females to be mated was 20 min. Copulations were not recorded during the experiment because of the movements of the animals and the difficulty of recognizing the marked wings with certainty.

At the end of each experiment (20 min), all the flies were fixed by pouring liquid nitrogen through the gauze cover. This was possible since copulation lasts more than 20 min and this method of fixation did not separate mates. The numbers given in the results take into account both directions of markings together.

Sexual isolation was calculated using Levene's index (Malogolowkin-Cohen et al. 1965). The isolation index is calculated as I = (sum of homogamic matings) − (sum of heterogamic matings) whose SE is given by the formula $[(1 - I^2)/N]^{1/2}$ where N is the total number of matings. The isolation index range is between +1 (complete isolation) and −1 (all matings are heterogamic) through zero (equivalent to panmixia).
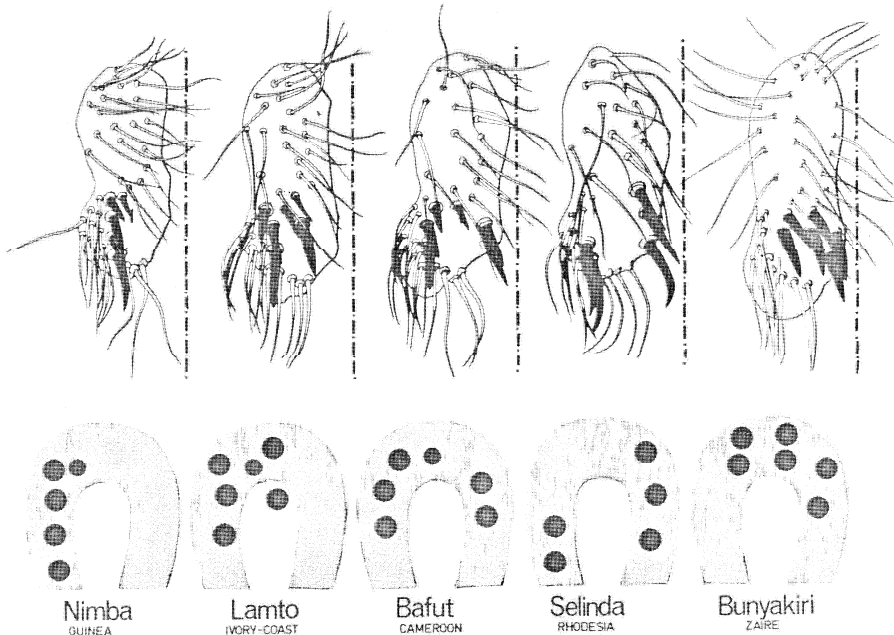
Fig. 1.—*upper*, Characteristic tooth patterns of the anal plates of male *Drosophila teissieri* from different locations in Africa. *lower*, Theoretical outlines of the displacement of the tooth row along the horseshoe pattern.

RESULTS

Morphological Analysis

Preliminary tests of visual recognition were made using two sets of 50 males from each strain mixed at random. They showed a discrimination of 100% between the furthermost populations (Nimba and Selinda). Between pairs of intermediary strains, the error reached 11% of the 100 individuals, i.e., a 22% risk. These tests showed striking discrimination, but they depended on the skill of the observer. A simple model was proposed to make it possible to measure the differences between the strains.

The arrangement of the teeth of the anal plates allows the assumption that they are arranged according to a "horseshoe" pattern (fig. 1). From Guinea to Rhodesia, the row of teeth moves from the external branch of the "horseshoe" to the internal one. In intermediate populations, the teeth are on the upper part. The biometrical analysis involved two variables which were the number of teeth on each branch of the left anal plate, as separated by a virtual line drawn between the lowest external tooth and the highest one, as shown in figure 2. Because of their convexity, the borders of the anal plates could not provide convenient landmarks.
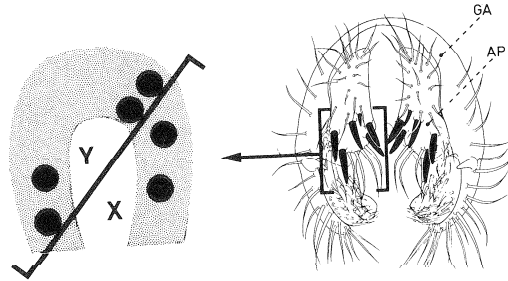
FIG. 2.—*left*, Arrangement of the line separating tooth sets involved in the biometrical analysis of the male anal plate variation in *Drosophila teissieri*. *right*, General features of male genitalia after Tsacas (1971). GA, genital arch; AP, anal plate.

The results are shown in table 1 by comparing the strains two by two on separate diagrams giving respectively the percentage of nonoverlapping values between the strains and the probability of discrimination, assuming that half of the individuals get the right determination at random.

The probability of discriminating between the furthermost populations (Guinea and Rhodesia) is about 95%. In intermediate strains, the greater the geographical distance, the greater the divergence. Thus, they show a clinal variation along their NW-SE general axis. Such difference between populations did not result from a genetic drift in the laboratory, since the two strains from Brazza, though collected 3 yr apart, yielded quite similar results with the other populations and showed a 99.5% similarity between them.

The sample of 29 individuals from Bunyakiri in Zaïre cannot be introduced in this analysis. Nevertheless, their tooth patterns were close to those from Selinda in Rhodesia.

In order to give a visual representation of this cline and the overlap between the populations, the values calculated in each strain for the two parameters are represented simultaneously by 95% probability ellipses in figure 3.

Chromosomal Polymorphism

Most strains studied (table 2) shared three polymorphic inversions on chromosome 2 ($2Lop/+$; $2Rf/+$; $2Rs/+$) and one on chromosome 3 ($3Lefg/+$). The strains from Makokou (Gabon), Lamto (Ivory Coast), Zoatoupsi (Cameroon) were homosequential. There was also no difference between the sequences of the strains from Selinda (Rhodesia) and Ozon (Cameroon). No endemic inversion was found in the eight strains studied from the Nimba mountains, contrary to what is found in the closely related species *D. yakuba* in which Nimba populations show endemic polymorphic sequences (Lemeunier and Ashburner 1976). However, none of the eight Nimba strains yielded the $2Rf/+$ inversion, though the latter is widespread in the other localities. This may indicate some incipient genetical divergence in this peripheral area.

TABLE 1

PROBABILITY OF DISCRIMINATION (D) AND OVERLAP (O) OF GEOGRAPHICALLY DISTANT POPULATIONS OF *D. teissieri* BY THE TOOTH PATTERN OF THE MALE ANAL PLATES

O \ D	Nim. 1,300 m	Nim. 720 m	Lam.	Baf.	N'Kol.	Mak.	Braz. 1974	Braz. 1977	Sel.
Nimba Guinea	···	96.5 ±2.6	78.0 ±5.8	35.5 ±6.8	30.5 ±6.5	40.5 ±6.9	47.0 ±7.1	47.0 ±7.1	16.5 ±5.2
Nimba Guinea	51.8 ±4.5	···	83.0 ±5.3	32.0 ±6.6	30.0 ±6.5	35.0 ±6.8	41.0 ±6.9	44.0 ±7.0	11.0 ±4.5
Lamto Ivory Coast	61.0 ±9.0	58.5 ±7.0	···	81.5 ±5.5	77.0 ±5.9	79.0 ±5.8	87.0 ±4.8	87.0 ±4.8	52.0 ±7.1
Bafut Cameroon	82.3 ±5.4	84.0 ±5.2	59.3 ±6.9	···	96.0 ±2.8	96.5 ±2.6	95.5 ±2.9	96.0 ±2.8	92.0 ±3.9
N'Kolbisson Cameroon	84.8 ±5.1	85.0 ±5.1	61.5 ±6.9	52.0 ±4.6	···	91.0 ±4.1	86.5 ±4.9	87.0 ±4.8	89.0 ±4.5
Makokou Gabon	79.8 ±5.7	82.5 ±5.4	60.5 ±6.9	51.8 ±4.5	54.5 ±5.8	···	93.5 ±3.5	93.0 ±3.6	95.5 ±2.9
Brazza 1974 Congo	76.5 ±6.0	79.5 ±5.7	56.5 ±6.8	52.5 ±4.8	56.8 ±6.9	53.3 ±5.2	···	99.5 ±0.8	86.5 ±4.9
Brazza 1977 Congo	76.5 ±6.0	78.0 ±5.9	56.5 ±6.8	52.0 ±4.6	56.5 ±6.8	53.5 ±5.3	50.3 ±3.7	···	83.0 ±5.3
Selinda Rhodesia	91.7 ±3.9	94.5 ±3.2	74.0 ±6.2	54.0 ±5.6	55.5 ±6.3	52.3 ±4.7	56.8 ±6.9	58.5 ±7.0	···

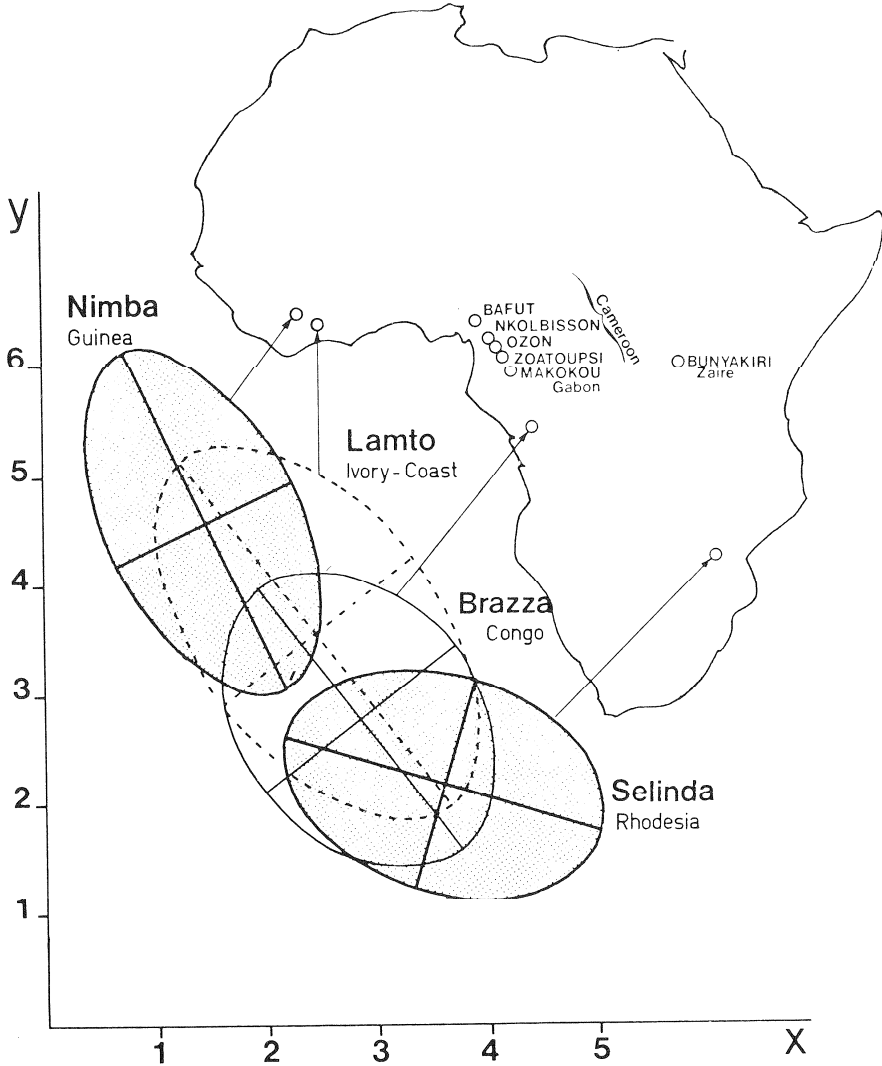NOTE.—Significant at the .05 level. Means in percentage and standard error.

FIG. 3.—Clinal differentiation of male anal plates in *Drosophila teissieri* throughout its range in Africa. Equal probability ellipses constructed on the major axis include 95% of the variability of the populations investigated. Ellipses of four populations are plotted here to clarify the figure. Those of other populations would be placed likewise in the axis of the cline in intermediate positions.

TABLE 2

DISTRIBUTION OF THE CHROMOSOMAL POLYMORPHISM IN *D. teissieri* INVERSIONS ON
CHROMOSOME 2 (*2L* AND *2R*) AND ON CHROMOSOME 3 (*3L*)

STRAIN	ORIGIN	ALTITUDE (m)		SEQUENCE OF INVERSIONS			
				2*Lop*	2*Rf*	2*Rs*	3*Lefg*
153	Nimba	1,300	Guinea	+	−	+	+
165.1	Nimba	1,300	Guinea	+	−	+	+
165.3	Nimba	1,300	Guinea	+	−	+	+
168	Nimba	850	Guinea	−	−	−	+
170	Nimba	820	Guinea	+	−	+	+
183	Nimba	810	Guinea	−	−	−	+
171	Nimba	720	Guinea	−	−	−	+
142	Nimba	650	Guinea	−	−	+	+
140.5	Lamto	80	Ivory Coast	+	+	+	+
144.4	Ozon	1,000	Cameroon	+	+	−	+
145.1	Zoatoupsi	1,000	Cameroon	+	+	+	+
131.3	Makokou	450	Gabon	+	+	+	+
128.2	Selinda	1,100	Rhodesia	+	+	−	+

Reproductive Isolation

No postmating isolation exists among the populations of *Drosophila teissieri*, since all the intercrosses between four strains (Nimba, Lamto, N'Kolbisson, Selinda) yielded hybrids which proved to be fertile in each kind of backcross. Each kind of mating was recorded in the behavioral tests, confirming the fact that these populations all belong to the same biological species. The values given by Levene's index at $P = .05$ are: Nimba × Lamto: $I = -.294 \pm .120$ ($\chi^2 = 1.67$; df = 3; nonsignificant at $P > .05$). Nimba × Brazza: $I = .000 \pm .441$ (−). Lamto × N'Kolbisson: $I = .000 \pm .294$ ($\chi^2 = 2.18$; df = 3; nonsignificant at $P > .05$). Lamto × Selinda: $I = .203 \pm .106$ ($\chi^2 = 4.08$; df = 3; nonsignificant at $P > .05$). Nimba × Selinda: $I = .320 \pm .263$ ($\chi^2 = 10.49$; df = 3; significant at $P < .01$).

The deviation from random mating was not significant in most cases and the panmictic hypothesis cannot be ruled out except in the experiments involving the furthermost populations, Nimba and Selinda, indicating a weak but statistically significant tendency for homogamy.

DISCUSSION

The populations of *Drosophila teissieri* collected from Guinea to Rhodesia all belong to the same species. The geographical variations they show in genitalia are gradual and their reproductive isolation very slight. However, the geographical origin of these populations can be roughly deduced from the tooth pattern of the anal plates in males. By using the common diagnostic character to distinguish *Drosophila* species, the furthermost populations may have been described as different species. Similar variation in tooth patterns might occur in *D. tsacasi* in Africa (L. Tsacas, personal communication) and in *D. funebris* in Europe (H.

Burla, personal communication), although the actual geographical basis of these morphological differences is unknown.

The situation found in *D. teissieri* is the opposite of that found in the neotropical *D. paulistorum* species complex, whose sibling species show no morphological differentiation (Pasteur 1970), yet they exhibit ethological isolation, hybrid sterility of males F_1 and complete reproductive isolation in the wild in sympatric conditions (Richmond and Dobzhansky 1976 and references therein). Ahearn et al. (1974) emphasize that ethological isolation and hybrid sterility are uncorrelated. In the special case of *D. teissieri*, it should be added that morphological differentiation in genitalia does not correlate with hybrid sterility. Despite the lack of any morphological differentiation, the speciation process has gone further in *D. paulistorum* and *D. birchii* (Ayala 1965; Baimai 1970) than in *D. teissieri*. In some cases, morphological divergence may then precede reproductive isolation in the speciation process. However, the available data are consistent with the general opinion that genitalia differentiation is correlated to genetic divergence. Curiously, a nascent, though slight behavioral isolation correlates with the most extreme morphological divergence between the extremities of the species range. This would contradict the general opinion (Mayr 1969; Dobzhansky 1970) that ethological differentiation originates in sympatric conditions as an ad hoc device to reinforce an isolation due to postmating barriers arising as incidental by-products of genetic divergence during a phase of allopatry. However, such a tendency to homogamy may be devoid of evolutionary significance as shown by Petit and al. (1976*a*, 1976*b*) in Japanese and French geographic strains of *D. melanogaster*.

LITERATURE CITED

Ahearn, J. N., H. L. Carson, Th. Dobzhansky, and K. Y. Kaneshiro. 1974. Ethological isolation among three species of the *planitibia* subgroup of Hawaiian *Drosophila*. Proc. Natl. Acad. Sci. USA 71:901–903.

Ashburner, M. 1967. Patterns of puffing activity in the salivary gland chromosomes of *Drosophila*. I. Autosomal puffing patterns in a laboratory stock of *Drosophila melanogaster*. Chromosoma (Berl.) 21:398–428.

Ayala, F. J. 1965. Sibling species of the *Drosophila serrata* group. Evolution 19:538–545.

Baimai, V. 1970. Additional evidence on sexual isolation within *Drosophila birchii*. Evolution 24:149–155.

Dobzhansky, Th. 1951. Genetics and the origin of species. 3d ed. Columbia University Press, New York.

————. 1970. Genetics of the evolutionary process. Columbia University Press, New York.

Ehrman, L. 1965. Direct observation of sexual isolation between allopatric and between sympatric strains of the different *Drosophila paulistorum* races. Evolution 19:459–464.

————. 1968. Reproductive isolation in *Drosophila*. Pages 85–87 *in* A. W. Stokes, ed. Animal behavior in laboratory and fields. Freeman, San Francisco.

Ehrman, L., and C. Petit. 1968. Genotype frequency and mating success in the *willistoni* species group of *Drosophila*. Evolution 22:649–658.

Elens, A. A., and J. M. Wattiaux, 1964. Direct observation of sexual isolation. Drosophila Info. Ser. 39:118–119.

Hollingsworth, M. J. 1960. The morphology of intersexes in *Drosophila subobscura*. J. Exp. Zool. 143:123–151.

Lemeunier, F., and M. Ashburner. 1976. Relationships within the *melanogaster* species subgroup of the genus *Drosophila* (Sophophora). II. Phylogenetic relationships between six species based upon polytene chromosome banding sequences. Proc. R. Soc. Lond., B. 193:275–294.

Malogolowkin-Cohen, Ch., A. S. Simmons, and H. Levene. 1965. A study of sexual isolation between certain strains of *Drosophila paulistorum*. Evolution 19:95–103.

Mayr, E. 1969. Principles of systematic zoology. McGraw-Hill, New York.

Pasteur, G. 1970. A biometrical study on the semispecies of the *Drosophila paulistorum* complex. Evolution 24:156–167.

Petit, C., O. Kitagawa, and T. Takamura. 1976*a*. Differenciation géographique et isolement sexuel: étude du mode de croisement entre lignées japonaises et françaises de *Drosophila melanogaster*. Arch. Zool. Exp. Gen. 117:345–358.

————. 1976*b*. Mating system between Japanese and French geographic strains of *Drosophila melanogaster*. Jpn. J. Genet. 51:99–108.

Richmond, R. C., and Th. Dobzhansky. 1976. Genetic differentiation within the Andean semispecies of *Drosophila paulistorum*. Evolution 30:746–756.

Snodgrass, R. E. 1957. A revised interpretation of the external reproductive organs of male insects. Smithson. Misc. Collect. 135:1–55.

Tsacas, L. 1971. *Drosophila teissieri*, nouvelle espèce africaine du groupe *melanogaster* et note sur deux autres espèces nouvelles pour l'Afrique. Bull. Soc. Entomol. Fr. 76:35–45.

Tsacas, L., C. Bocquet, M. Daguzan, and A. Mercier. 1971. Comparaison des génitalia mâles de *Drosophila melanogaster*, de *Drosophila simulans* et de leurs hybrides (Dipt. Drosophilidae). Ann. Soc. Entomol. Fr. 7:75–93.

Wiley, E. O. 1978. The evolutionary species concept reconsidered. Syst. Zool. 27:17–26.

D. LACHAISE
F. LEMEUNIER
M. VEUILLE

LABORATOIRE DE BIOLOGIE ET GÉNÉTIQUE
EVOLUTIVES DU C. N. R. S.
91190 GIF SUR YVETTE
FRANCE