EVOLUTION OF THE OBSCURA GROUP DROSOPHILA SPECIES. I. SALIVARY CHROMOSOMES AND QUANTITATIVE CHARACTERS IN *D. SUBOBSCURA* AND TWO CLOSELY RELATED SPECIES*

C. B. KRIMBAS AND M. LOUKAS

Department of Genetics, Agricultural College of Athens, Greece.

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SUMMARY

Three closely related *Drosophila* species of the "obscura" group have been examined: *Drosophila madeirensis*, *D. guanche* and *D. subobscura*. Some crosses are possible between them and F_1 progeny were used in order to clarify chromosomal homologies. *D. madeirensis* has the following constitution in relation to *D. subobscura*: A_{m+1} ; J_{ST} ; E_{ST} ; O_3 ; U_{1+2} while *D. guanche* has: A_{m+g} ; J_g ; E_g ; O_{3+g} ; U_{1+2} (where m and g indicate inversions specific to those species). Some data are also presented on the inheritance of eight different quantitative characters used by some taxonomists to differentiate other palearctic obscura group species.

1. INTRODUCTION

Several species belonging to the "obscura" group of the genus *Drosophila* have been extensively used in population genetic and speciation studies. Thus the American species *D. pseudoobscura* and *D. persimilis* were the favourite material of Th. Dobzhansky and his school while the palearctic *D. subobscura* was used by the European workers. Several other American species of the *affinis* sub-group were also studied. The information, however, concerning the evolution of all the 32 species belonging to this group and known until now, is sparse and consists mainly of the construction of phylogenetic trees based on electrophoretic data. We intend in this series of papers to present more data on the evolution of obscura group species and especially of the palearctic ones. The present paper deals with the chromosomal homologies between *D. subobscura*, *D. guanche* and *D. madeirensis*: homologies have been traced mainly by synaptic relations observed in F_1 hybrids. Some indications are also provided concerning the inheritance of eight quantitative characters that taxonomists use in order to distinguish obscura group species.

2. MATERIALS

A strain of *Drosophila guanche* Monclús (1976) and a strain of *D. madeirensis* Monclús, kindly provided by Professor Antonio Prevosti, were used in this study. *D. guanche* originated from the Canary Islands where it is confined and *D. madeirensis* is native to the island of Madeira.

* This paper is dedicated to Professor Ernst Mayr for his 80th birthday. Besides being a leading evolutionist and one of the founders of the synthetic theory, he also propounded and clarified the antiplatonic populational way of looking at the world: "φίλος μὲν Πλάτων, φιλάττη δὲ ἡ ἀλήθεια".

Three strains from *D. subobscura* were used in this study: the Kusunacht strain monomorphic for the Standard gene arrangements in all its chromosomes (A_{ST} , E_{ST} , J_{ST} , O_{ST} , U_{ST}), the *ch cu* strain (a strain homozygous for the cherry eyes and curled wings recessive mutations and for the gene arrangements A_{ST} , E_{ST} , J_{ST} , O_{3+4} and U_{ST}) and finally the strain No. 8 from Crete having the following gene arrangements: A_2 , E_{ST} , J_{ST} , O_{3+4} , U_{1+2} .

All measurements of lengths, teeth, chaetae were performed under microscopic observation of preparations of the respective parts of the fly mounted in euparal.

3. SPECIES CROSSES AND HYBRIDS

Crosses in all directions cannot be performed. Only two successful crosses yield adult progeny and are the following:

(i) ♀ *D. madeirensis* × ♂ *D. guanche*. This yields a small number of F_1 female and male flies both of which are sterile. Thus an F_2 and backcrosses to parents cannot be obtained.

(ii) ♀ *D. madeirensis* × ♂ *D. subobscura*. The F_1 males are sterile, but F_1 females are fertile and can backcross to both parents. These two crosses were obtained relatively easily when the strains of *D. guanche* and *D. madeirensis* first arrived in our laboratory. It proved, however, more difficult to repeat them some months later on.

4. THE MITOTIC CHROMOSOMES

It is well known that *Drosophila subobscura* displays twelve chromosomes in complete somatic mitosis: two dots and five pairs of rods in females. One of these pairs consists of the sex chromosomes.

Squashes from larval nervous ganglia of *D. guanche* have also revealed twelve chromosomes; two dots and five pairs of rod-like chromosomes (chromosomes of one or two pairs could bear a small, heterochromatic? second arm). Some individuals display an unequal pair while others do not. The unequal pair are apparently the sex chromosomes, the Y being a smaller, nearly dot-like, chromosome.

Dots are rather small and could be easily missed. They are not seen in similar microscopic preparations of *D. madeirensis*, but this is not a final proof of their absence. Ten chromosomes, forming three pairs of rods and two pairs of bigger rod-like or perhaps slightly acrocentric chromosomes with a smaller (heterochromatic?) arm are seen clearly.

Thus there is a good correspondence between the mitotic chromosomes of these three closely related species.

5. SALIVARY GLAND CHROMOSOME HOMOLOGIES BETWEEN *D. MADEIRENSIS* AND *D. SUBOBSCURA*

The A (=X) chromosome of the F_1 female hybrids between *D. madeirensis* and *D. subobscura*, when a *ST* gene arrangement strain of this last species is used in the cross, shows two distantly located inversions (plate 1a). The first is a small one, located at the distal end of this chromosome (the free end) and involves the terminal section 16 BCD, which is

PLATE 1. The A (=X) chromosome in female F₁ hybrids from the cross *D. madeirensis* × *D. subobscura*.

inverted in *D. madeirensis*. It is the only specific inversion of *D. madeirensis* found in this material and it seems that our strain of *D. madeirensis* is monomorphic for it. The synapsis in an inversion heterozygote, an F_1 hybrid, is clearly seen in plate 1b, where after synapsis the two homologous chromosomes depart unpaired to the left, thus permitting a perfect match of their distal ends. The second inversion is a longer one located near the proximal end of the chromosome, the end near to the centromere. Plate 1c shows clearly the distal break point located near 7C. We feel that it is an identical inversion to A_1 , one found in several natural populations of *D. subobscura* and for which our *D. madeirensis* strain seems to be monomorphic. A final proof of the identity will be provided when *D. madeirensis* will be crossed to an A_1 strain of *D. subobscura*.

The *E* chromosome of *D. madeirensis* in our strain shows a perfect synapsis with the E_{ST} of *D. subobscura* along nearly all its length (plate 2a). After the section 57 (which is the usual landmark for *E*) and towards the centromere end (at the beginning of 54), the homologous chromosomes systematically do not synapse although the banding pattern is more or less similar: the asynapsis is probably due to minor differences. In the same figure a complete synapsis can be observed in the dot (section 100).

A perfect synapsis is also observed in most parts of the *J* chromosome from section 34 to section 20 inclusive, in the F_1 hybrids when the *subobscura* chromosome is of the *ST* gene arrangement (plate 2b). The free end (section 35) shows a partial asynapsis, perhaps due to minor differences, but the banding pattern seems similar. In the same figure the dot and the proximal part of the *E* chromosome are also shown. In plate 5b the synapsis of the sections 19 to 17 of the *J* chromosome (part near the centromere) is nearly perfect. A simple inversion is seen near the distal end of chromosome *O* in F_1 hybrids when the *subobscura* parent strain is monomorphic for the O_{3+4} gene arrangement (plates 3a and 4), while another simple inversion, but further from the distal end, is observed when the *subobscura* parent has the O_{ST} gene arrangement (plate 3b). It is quite evident that our *D. madeirensis* strain has an O_3 inversion in its *O* chromosome. This is the hypothetical missing link between the O_{ST} gene arrangement and that produced by the two overlapping inversions O_{3+4} . It was not found until now in any of the nearly hundred natural populations of *D. subobscura* studied (Krimbas and Loukas 1980 for a review, and Sperlich *et al.*, 1981, Larruga *et al.*, 1983, de Frutos pers. com. for more recent data not included in this review) and was supposed to have been eliminated according to the triad rule formulated by Bruce Wallace (1953). It has been, however, preserved in *D. madeirensis* (and in *D. guanche*, as will be mentioned below). In plates 3a, 4 and 3b the landmarks are also indicated corresponding to those of the map of Kunze Mühl and Müller (1958). In plates 3a and 4 the combination shown is O_3/O_{3+4} which displays the simple inversion loop O_4 on a homozygous O_3 background, while in plate 3b the combination shown is O_3/O_{ST} which displays the simple inversion loop O_3 on a homozygous O_{ST} background. Apart from that, the *O* chromosomes of the two species seem to pair nearly perfectly—there are some small banding differences, such as bands missing etc., in the sections 89, 87, perhaps 83, 79, 78 and especially in 76 and 75. Plate 5b, at the right, shows a O_3/O_{3+4} inversion and partial asynapsis near the centromere end of the *O* chromosome.

The combination of the *U* chromosome of *D. madeirensis* in our strain with a U_{ST} of *D. subobscura* shows the two characteristic and contiguous (but not overlapping) inversions U_{1+2} (plates 6a and 6b). That the *U* chromosome of *D. madeirensis* from our strain has the gene arrangement U_{1+2} is proved in plate 5a, which displays the chromosomes of the F_1 hybrid between *D. madeirensis* and strain No. 8 of *D. subobscura*, a strain monomorphic for the U_{1+2} gene arrangement. There are no inversions and there is a perfect synapsis from section 53 towards the other chromosome end. This is shown in the middle of the figure. At the left of the same figure we can observe the perfect synapsis of the *E* chromosome, while running horizontally we can see the proximal end of the *O* chromosome (with the characteristic asynapsis in sections 76 and 75) as well as the proximal end of the *J*, which in this figure is completely asynaptic with one of the two chromosomes broken at section 20. Finally, at the upper part of this photograph and also repeated at the right upper corner, the *A* chromosome is seen with the small distal inversion together with the two simple inversions A_2 and A_1 (strain No. 8 is monomorphic for the A_2 gene arrangement).

In conclusion, our strain of *D. madeirensis* has, except for the small distal inversion on chromosome *A* and some minor differences in other chromosomes, the following constitution of gene arrangements with respect to *D. subobscura*:

$$A_1, E_{ST}, J_{ST}, O_3, U_{1+2}$$

6. SALIVARY GLAND CHROMOSOME HOMOLOGIES BETWEEN *D. MADEIRENSIS* AND *D. GUANCHE*

General views of the salivary gland chromosomes of *D. guanche* are shown in plates 7 and 8. In these figures we indicate also an interpretation of the chromosomal sections in relation to those of *D. subobscura*. Thus plate 7 shows in its upper part the distal part of the *J* chromosome, below it the entire *O* chromosome, which has a gene arrangement O_3 with a superimposed (overlapping) inversion specific to *D. guanche* covering the sections 85 to 91AB plus 94E. Below that, in the middle, the chromosome *A* is seen which retains the small distal inversion of *D. madeirensis*, namely the one involving the section 16 *BCD*, and also is monomorphic for an inversion specific to this species including the sections 9 to 14, and also does not have the inversion A_1 of *D. madeirensis* but instead bears the *ST* gene arrangement. Below these the chromosomes *E* and *U* are shown. *E* has the *ST* gene arrangement with a specific inversion of this species including the section 71AB to 73. On the other hand, *U* has the U_{1+2} gene arrangement. The dot is shown at the left between the centromere ends of *E* and *U* chromosomes. The same is shown in plate 8 but here we can also observe completely the *J* chromosome which bears the *ST* gene arrangement with an inversion specific to *D. guanche* involving the sections 34A to 31.

The *U* chromosome of F_1 hybrids between *D. madeirensis* and *D. guanche* is shown in plate 9b. Although the pairing is not perfect the homologies are clear: *guanche* has a *U* chromosome of the 1+2 gene arrangement. Plate 9c shows the incomplete pairing of the same F_1 hybrids for the *J* chromosome. The inversion specific to *guanche* is also clearly depicted. Plate 9a shows the pairing of the *E* chromosome in the F_1 hybrids.

PLATE 2. The *E* (2a) and *J* (2b) chromosomes in *D. madeirensis* × *D. subobscura* F₁ hybrids.

PLATE 3. The *O* chromosome in *D. madeirensis* × *D. subobscura* F_1 hybrids. In 3a, the *D. subobscura* parent was homozygous for the gene arrangement O_{3+4} , where in 3b it was homozygous for O_{ST} .

PLATE 5. Chromosomes of *D. madeirensis* $\times$ *D. subobscura* F_1 hybrids. Further explanation in text.

PLATE 4. The terminal end of the *O* chromosome in a *D. madeirensis* × *D. subobscura* F_1 hybrid, where the *D. subobscura* parent was homozygous for the O_{3+4} gene arrangement. The inversion O_4 is shown.

PLATE 6. The *U* chromosomes of *D. madeirensis* × *D. subobscura* F₁ hybrids.

PLATE 7. The salivary chromosome of *D. guanche*.

PLATE 8. The salivary chromosomes of *D. guanche*.

PLATE 9. The E (9a), U (9b), and J (9c) chromosomes in F₁ hybrids from the cross *D. madeirensis* × *D. guanche*.

PLATE 10. The *O* chromosome in *D. madeirensis* $\times$ *D. guanche* F_1 hybrids.

There is practically no synapsis except for the distal end and the inversion specific to *guanche*.

Finally in plate 10 we can see the pairing of the *O* chromosome in *madeirensis* $\times$ *guanche* F_1 hybrids. It is not clear whether section 84 exists in *D. guanche*. However, the inversion specific to *D. guanche* includes 94E until the beginning of 85. The asynapsis is nearly general but it seems that banding patterns, although somewhat different in details, are very similar.

There is no figure depicting the *A* chromosome of the hybrids. It seems that natural populations of *D. guanche* do not show inversion heterozygosity (Cabrera, pers. com.). Thus the results obtained with our strain have a general application.

In conclusion, *D. guanche* has the following gene arrangements in comparison to the *ST* ones of *D. subobscura*: *A*(inv 16BCD) + (inv 9-14); *E*(inv 71AB-73); *J*(inv 31-34A); *O*(inv 3) + (inv 85-91AB + 94E); U_{1+2} .

D. guanche has four specific inversions plus two in common with *D. madeirensis*. It differs from this one by having an A_{ST} (instead of A_1) gene arrangement but otherwise is more similar to *D. madeirensis* than to *D. subobscura*.

7. NOTES ON THE INHERITANCE OF SPECIES SPECIFIC QUANTITATIVE CHARACTERS

Burla (1951) devised and used several indexes permitting differentiation of the European species belonging to the *obscura* group known at that time: the ratio of lengths of the first two tarsal segments, the number of teeth in the claspers of the external male genital apparatus, the chaetae on the female ovipositor guide, the number of teeth in the male sex combs as well as four wing indexes, ratios of lengths of different parts of the wing. These eight characters have been measured in the parental species, their F_1 hybrids and, in the case of *D. madeirensis* $\times$ *D. subobscura* matings, in backcross progeny of the F_1 with the *subobscura* male parent. About ten such backcross families were raised and a total number of 88 males and 113 females from this backcross were measured. Not all characters, however, have been measured in all F_1 and backcrosses.

(i) *Ratio of the lengths of the tarsal segments (Ist/IIInd)*

The mean of the three species under consideration differ and even the individual values nearly do not overlap. Only the F_1 of the *madeirensis* $\times$ *subobscura* cross has been measured: the mean value coincides with that of the *subobscura* parent, which thus displays complete dominance. No backcrosses were measured (table 1).

(ii) *The sum of the number of teeth in both male claspers (forceps)*

The means of *madeirensis* and *subobscura* differ but the distributions greatly overlap. The F_1 mean does not differ from *madeirensis* ($t = 1.02$) but does so from *subobscura*. This suggests that the dominant alleles are located in *madeirensis*. However the B_2 mean shows a complete reversion to that of *subobscura* thus suggesting that the simultaneous presence of more than one factor from *madeirensis* is needed in order to get an increased number of teeth. This kind of epistatic interaction permits only a few

TABLE 1

Ratio of the lengths of the two tarsal segments. n = number of individuals measured, m = mean value and l = extreme measurements observed in our sample

	<i>madeirensis</i>	<i>subobscura</i>	<i>guanche</i>	F_1 (<i>madeirensis</i> $\times$ <i>subobscura</i>)
n	33	23	19	26
m	1.08 ± 0.009	1.27 ± 0.010	1.43 ± 0.018	1.27 ± 0.016
l	0.97–1.18	1.18–1.34	1.28–1.58	1.12–1.38

TABLE 2

Teeth in male claspers. v = variances, other symbols as in Table 1

	<i>madeirensis</i>	<i>subobscura</i>	F_1 (<i>madeirensis</i> $\times$ <i>subobscura</i>)	B_2 (F_1 $\times$ <i>subobscura</i>)
n	20	20	22	88
m	13.55 ± 0.22	12.00 ± 0.10	13.82 ± 0.14	11.48 ± 0.09
v	0.997	0.205	0.442	0.794
l	12–15	11–13	13–15	10–15

TABLE 3

The observed distributions of the number of teeth in male claspers. The numbers indicate the individuals observed

	number of teeth					
	10	11	12	13	14	15
<i>madeirensis</i>			4	4	9	3
<i>subobscura</i>		2	16	2		
F_1				7	12	3
B_2	3	9	58	9	8	1

individuals from the B_2 to have high teeth number and thus to resemble *madeirensis* (table 2). Table 3 gives details of the observed distributions. The small variance of the *subobscura* parent is probably due to the lack of genetic heterogeneity in the strain measured. The B_2 distribution shows a lack of symmetry due to the 6 individuals (five with 14 teeth and one with 15) which display high teeth numbers. Without them the B_2 mean could be exactly 12.00 as in the *subobscura* parent. If these 6 individuals are considered to display the *madeirensis* phenotype, then the presence of 6.8 per cent of these individuals would suggest the segregation of some 4 independent effective factors (the value expected would be 6.25 per cent). Further investigation is planned to clarify this point, and whether chromosomes can be identified as effective factors.

D. guanche has a mean number of teeth 16.28 (7.96 in one clasper 8.32 in the other) while the 13.55 of *madeirensis* splits accordingly as 6.59 and 6.95. Only one F_1 (*madeirensis* $\times$ *guanche*) was measured in one clasper and showed 8 teeth thus resembling the *D. guanche* parent.

(iii) Mean number of chaetae on the female ovipositor guide (vaginal plates)

This is the mean of the left and right parts. *Madeirensis* has a different mean from the other two, while the distributions of *subobscura* and *guanche*

TABLE 4
Mean number of chaetae on the ovipositor guide

	<i>madeirensis</i>	<i>subobscura</i>	F_1 (<i>madeirensis</i> × <i>subobscura</i>)	B_2	<i>guanche</i>	F_1 (<i>madeirensis</i> × <i>guanche</i>)
<i>n</i>	22	22	23	113	24	1
<i>m</i>	19.13 ± 0.29	15.25 ± 0.29	15.43 ± 0.31	15.27 ± 0.16	16.60 ± 0.19	15.5
<i>v</i>	1.894	1.804	2.211	2.889	0.847	—
<i>l</i>	16.5–22.5	12.5–17.5	12–17.5	12.5–18	15.5–18.5	—

TABLE 5(a)
Number of teeth in the sex combs of both tarsal segments for the first and the second one

	<i>madeirensis</i>	<i>subobscura</i>	<i>guanche</i>	F_1 (<i>madeirensis</i> × <i>subobscura</i>)	B_2	F_1 (<i>madeirensis</i> × <i>guanche</i>)
<i>n</i>	35	23	44	23	88	1
<i>m</i>	31.51 ± 0.46	20.7 ± 0.18	49.84 ± 0.39	26.57 ± 0.33	22.29 ± 0.26	40
<i>l</i>	26.36	19–23	42–55	23–30	17–29	—
<i>v</i>	7.316	0.747	6.555	2.439	5.922	—
$I_{st} \left\{ \begin{matrix} m \\ l \end{matrix} \right.$	17.69 ± 0.29 14–21	10.48 ± 0.20 9–12	27.71 ± 0.24 23–31	14.17 ± 0.23 12–16	11.28 ± 0.18 9–15	21 —
$II_{nd} \left\{ \begin{matrix} m \\ l \end{matrix} \right.$	13.83 ± 0.27 11–16	10.26 ± 0.13 9–12	22.14 ± 0.24 19–26	12.39 ± 0.22 10–14	11.01 ± 0.14 8–16	19 —

greatly overlap. The F_1 mean is different from *madeirensis* but not from *subobscura* and the B_2 mean (it is actually the mean of ten families) is similar to F_1 and to *subobscura* (table 4). The only F_1 measured between *madeirensis* and *guanche* resembles the *guanche* parent.

(iv) *The number of teeth in the sex combs of the first and second tarsal segments added*

All three species differ in this number and distributions do not overlap. This is mainly due to the number of teeth on the proximal to the body tarsal segment, the first one (table 5(a)). There is no directional dominance: The F_1 mean of the *madeirensis* $\times$ *subobscura* cross does not differ from the mid parent value (26.125), while the B_2 is slightly greater than the expected without dominance. The other F_1 (*madeirensis* $\times$ *guanche*) seems to follow the same trend, but this is suggested by the measurement of one individual only.

TABLE 5(b)

Data derived from those of Table 5b after a scale transformation. Further explanations in the text

	<i>madeirensis</i>	<i>subobscura</i>	F_1 (<i>madeirensis</i> $\times$ <i>subobscura</i>)	B_2 (F_1 $\times$ <i>subobscura</i>)
<i>n</i>	35	23	23	88
<i>m</i>	1.252	0.857	1.113	0.928
<i>v</i>	0.0047	0.0026	0.0029	0.0151

Since it seems that in the case of the total number of teeth in the sex combs we have a near additivity we can try to estimate the number of genes using the formula given in Wright (1968, p. 394):

$$n = \frac{(\bar{P}_2 - \bar{F}_1)^2}{4(\sigma_{B_2}^2 - \sigma_E^2)}$$

where n is the number of genes controlling the character, $\bar{P}_2$, $\bar{F}_1$ the respective means of *D. subobscura* and the F_1 hybrid, $\sigma_{B_2}^2$ the variance of the backcross and σ_E^2 the environmental variance. This last variance could be estimated as the mean of the variances of the *D. subobscura* parent (which is highly homozygous) and of the F_1 . However, it seems that there is an obvious dependence of the environmental variance on the mean. A scale transformation suggested by Wright (1968, p. 232) is the following

$$x' = \log(x - 13.488)$$

where x' are the values of x in the new scale. Table 5(b) provides the estimations of means and variances after scale transformation. The estimation of $\sigma_E^2 = 0.00276$ (the mean of $\sigma_{F_1}^2$ and $\sigma_{P_2}^2$).

Thus

$$n = \frac{0.065}{0.049} = 1.3.$$

The number of genes involved seems to be between one and two. However this could also mean that no crossing over takes place in F_1 females (this is apparently the case for chromosome *U* and partly for *O* and *A* at

least because of inversion heterozygosity) and that the genes controlling the character are located in one or two segregating chromosomes. Further data will clarify whether the actual gene number is so small as one suggested by the punctuated-macromutation theory.

It is interesting to note that our data permit us also to calculate an environmental-intraindividual variance since in 74 cases both the right and the left feet sex combs were measured. This variance turns out to be equal to 0.00253 (the total environmental variance being 0.00276). Thus 92 per cent of the total environmental variance is due to intraindividual developmental noise. Nearly the same percentage (97 per cent) of total environmental variance was found to be due to an intraindividual variance in the case of abdominal bristle number in *Drosophila melanogaster* (Reeve and Robertson, 1954).

(v) *Wing indexes*

Four wing indexes have been considered: First, the costal index (C.I.) which is the ratio of the length of the second section of the costal vein to that of its third section. Second, the fourth vein index (4v.I) which is the ratio of the lengths of the distal to the median section of the fourth vein. Third, the 4c index which is the ratio of the third costal section to that of the median section of the fourth vein. Finally, the 5x index which is the ratio of the last section of the fifth vein to that of the posterior cross vein. (table 6). All distributions vastly overlap except perhaps for the 4c index of *madeirensis* which differentiates this species from the other two. The F_1 of *madeirensis* $\times$ *subobscura* resembles *madeirensis* for the costal index (but on the contrary the F_1 of *madeirensis* $\times$ *guanche* shows an absence of dominance), and the same is true for the 4x index (and this in both F_1 's), and also for the 4c index (but the F_1 of *madeirensis* $\times$ *guanche* displays an absence of dominance). The 5x index differs in this respect: The F_1 resembles the *subobscura* parent.

In conclusion, when *madeirensis* and *subobscura* are crossed, in one character there is absence of dominance, in three the *subobscura* parent shows dominance over *madeirensis* and in the other four, the *madeirensis*. The F_1 of *madeirensis* and *guanche* shows absence of dominance in three characters, in one the *madeirensis* phenotype dominates and in two the *guanche* one.

8. DISCUSSION

The three species considered in this paper are apparently closely related and this can also be seen from the examination of their salivary gland chromosomes and those of their F_1 hybrids. Thus *D. madeirensis*, except for a small inversion in the distal part of the A chromosome, has a constitution that does not differ from known gene arrangements of *D. subobscura*. The case of chromosome O confirms this rule: it is evident that, at some time, inversion O_3 was present in this species since the other two arrangements, O_{5T} and O_{3+4} , now abundant in its natural populations, necessitated its presence. *D. guanche*, although in many aspects more similar to *D. madeirensis* than to *D. subobscura*, displays an array of inversions specific to this species. The suggested phylogeny from the study of gene arrangements, a first dichotomy giving *D. guanche*, and a later one of the other

TABLE 6
Inheritance of the four vein indexes. Mean values are indicated

	<i>madeirensis</i>	<i>subobscura</i>	<i>guanche</i>	F_1 (<i>madeirensis</i> $\times$ <i>subobscura</i>)	F_1 (<i>madeirensis</i> $\times$ <i>guanche</i>)
C.I.	2.95 $\pm$ 0.03	2.49 $\pm$ 0.014	2.53 $\pm$ 0.01	2.98 $\pm$ 0.02	2.74 $\pm$ 0.17
<i>I</i>	2.64-3.33 (<i>n</i> = 46)	2.20-2.61 (<i>n</i> = 40)	2.43-2.75 (<i>n</i> = 47)	2.76-3.25 (<i>n</i> = 49)	2.43-3.02 (<i>n</i> = 4)
4v.I.	1.80 $\pm$ 0.02	2.20 $\pm$ 0.02	2.20 $\pm$ 0.02	1.87 $\pm$ 0.01	1.89 $\pm$ 0.05
<i>I</i>	1.58-2.16 (<i>n</i> = 44)	1.93-2.36 (<i>n</i> = 40)	1.93-2.45 (<i>n</i> = 47)	1.52-2.00 (<i>n</i> = 50)	1.79-2.00 (<i>n</i> = 4)
4c.I.	0.86 $\pm$ 0.009	1.11 $\pm$ 0.008	1.13 $\pm$ 0.006	0.86 $\pm$ 0.007	0.93 $\pm$ 0.04
<i>I</i>	0.75-1.00 (<i>n</i> = 46)	1.03-1.23 (<i>n</i> = 40)	1.02-1.20 (<i>n</i> = 47)	0.71-0.95 (<i>n</i> = 50)	0.86-1.00 (<i>n</i> = 4)
5v.I.	1.38 $\pm$ 0.02	1.77 $\pm$ 0.02	1.87 $\pm$ 0.01	1.70 $\pm$ 0.02	not recorded
<i>I</i>	1.11-1.67 (<i>n</i> = 46)	1.40-2.05 (<i>n</i> = 38)	1.62-2.07 (<i>n</i> = 43)	1.30-2.01 (<i>n</i> = 49)	— —

branch *D. madeirensis* and *D. subobscura*, is the same suggested by the morphological differences between these three species as well as the one deduced from the electrophoretic data, as the second paper of this series exemplifies. A word should be added on the antiquity of inversions and especially on the original gene arrangements. Krimbas and Loukas (1980), Pinsker and Sperlich (1981) and other workers during the 1981 second *Drosophila subobscura* meeting in Barcelona tried to detect the original gene arrangements of this species based mainly on three different criteria: firstly on the centrality of the position in the phylogenetic tree (they actually followed that in Olvera *et al.* (1979), which is in a sense the usual practice used in converting an unrooted tree to a rooted one e.g., by the UPG method), secondly on the data provided by the actual geographic distribution of gene arrangements (the more widely distributed is inferred to be the more ancient), thirdly on the genic polymorphism of genes included within the inversion (the higher the number of effective alleles the more ancient the gene arrangement is). Fig. 1 displays seven gene arrangement phylogenies (chromosomes A and O are considered as composed of two independent segments). By arrows we indicated the middle positions between the most distant arrangements found (every arrangement differs from another by one step: a simple inversion) and encircled are the ones found in *D. guanche* and *D. madeirensis*. In table 7 are summarised the

TABLE 7

Gene arrangements inferred as original using different criteria by different authors

Chromosome	Criterion of centrality	Krimbas and Loukas' (1980) suggestion	Pinsker and Sperlich (1981) suggestion	Prevosti's suggestion (pers. com.)	Gene arrangements found in the two closely related species
A segment I	A_5	A_{ST}	—	—	A_1, A_{ST}
A segment II	A_2	A_2	—	—	A_{ST}
		(or derivative)			
J	J_{ST} or J_3	J_1	—	—	J_{ST}
U	U_{1+2}	U_{1+2}	—	—	U_{1+2}
E	E_{1+2}	E_{1+2}	—	—	E_{ST}
O segment I	O_3	O_{3+4}	O_{ST}	O_{ST}	O_3
O segment II	O_{ST}	O_{ST}	—	—	O_{ST}

original arrangements hypothesised by different authors using different criteria as well as the arrangements found in the two closely related species examined. Krimbas' and Loukas' (1980) predictions depart from the ones that a simple centrality criterion would indicate, in three out of seven instances: a second concomitant criterion is also used by them, that of geographic distribution. Prevosti's prediction is based exclusively on the third criterion. If the data provided by *D. madeirensis* and *D. guanche* could be considered to shed some light on this problem it seems that only one out of the five predictions of Krimbas and Loukas (1980) is correct. Indeed the actual geographic distribution is a pattern originating much later and seems to be a poor indicator of antiquity. But centrality also, although

FIG. 1. Unrooted phylogenies for gene arrangements in *Drosophila subobscura*. They are almost identical to those already published by Krimbas and Loukas (1980), except for inversion O_{16} which is omitted as found identical to O_{23} (Pinsker and Sperlich, 1981) and E_{16} which is added (Sperlich, Pinsker and Mitrophanov, 1981). Further explanations in the text.

providing better predictions, seems to lead to wrong answers in three out of seven cases! It is a criterion of a rather dubious value. On the other hand the observation that O_{ST} has more genetic heterogeneity than O_{3+4} in the inverted segment can now be clarified when the original arrangement is neither the first or the second but the "then hypothetical" O_3 . Another hypothesis to suffer from these results is the one advanced by Zapata, Santos and Alvarez (1982) which explains the coexistence of triads, contrary to the Wallace's rule, and predicts at the same time the original arrangements of a triad. Their predictions were nearly identical to those of Krimbas and Loukas (1980), that is to say they considered as original the gene arrangements U_{1+2} , E_{1+2+9} and O_{3+4} . From the data presented here, only the first of them could be considered correct.

The case of finding a hypothetical missing link in an inversion phylogeny is interesting per se. First it provides strong evidence in favor of the principles used in order to construct gene arrangement phylogenies (inversions are very rare, unique or historical events in the history of species) and also constitutes a further proof of the evolutionary process in general. This is not, of course, the first case of such a scientific finding, Dobzhansky having already encountered identical situations in *D. pseudoobscura* and *D. azteca* (Dobzhansky and Sturtevant, 1938; Dobzhansky, 1941).

The differences of genetic constitution of closely related species, and especially on those variants that control quantitative characters, has recently received an increased interest and many of the various mechanisms explaining speciation events could be clarified when relevant data accumulate. We have concentrated our attention on biometrical characters and indexes used to distinguish other palearctic species belonging to the *obscura* group. The data, however, are still scanty, partly because the material itself does not allow a complete analysis (an F_3 is impossible). It is, however, hoped that soon more data, based on chromosomal substitutions, will provide more information on this matter.

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