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Evolutionary cytogenetics of the *Drosophila buzzatii* species complex

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The salivary gland chromosomes of 10 species in the *Drosophila mulleri* subgroup (*repleta* group) have been re-analysed. These include the eight members of the South American *buzzatii* and *martensis* clusters, previously ascribed to the *mulleri* complex, and the two Caribbean species *D. stalkeri* and *D. richardsoni*, previously comprising the *stalkeri* complex. The chief results can be summarized as follows. Inversion 3a is not present in the *martensis* cluster. Hence, there is no cytological link between this cluster, or the *buzzatii* cluster, and the rest of the *mulleri* complex. Accordingly, a new species complex, the *buzzatii* complex, is established with the two South American clusters. *D. stalkeri* and *D. richardsoni* share at least two inversions with all the species in the *buzzatii* and *martensis* clusters, and produce hybrids in interspecific crosses with many of them. This indicates a close phylogenetic relationship. Therefore, *D. stalkeri* and *D. richardsoni* are incorporated as a cluster within the newly erected *buzzatii* complex. A phylogenetic tree illustrating the chromosomal evolution of the *buzzatii* complex is presented and all the previous cytological information concerning its members is reviewed.

Keywords: chromosomal evolution, *Drosophila repleta* species group, paracentric inversions, phylogeny.

Introduction

Changes in the number and shapes of chromosomes, resulting from reciprocal translocations, centric fissions and fusions, inversions, and additions and deletions of heterochromatin, have been used to determine phylogenetic relationships for many years (White, 1948). The rediscovery of the giant salivary chromosome with its somatic pairing of homologs in many of the species of the Diptera has resulted in an increase in the resolving power of at least two orders of magnitude above even the most modern banding techniques used in the study of mitotic chromosomes (Clayton & Guest, 1986).

Sturtevant & Dobzhansky (1936) showed that overlapping paracentric inversions can show phylogenetic relationships, but not the direction of evolution. Wasserman (1963, 1992) stated that each inversion is probably not a unique event but can occur more than once in more than one population. However, he argued that the probability of the occurrence and survival of the same inversion in two independent evolutionary lines is so minute that one can assume that each inversion is unique and that any two species which have the same inversion are more closely related to each other than either is to a third species which lacks the inversion. Wasserman (1963, 1992) also discussed the types of errors that could be made in this type of work. These included observational mistakes, and errors due to parallel and convergent evolution. Observational errors are relatively uncommon, but unfortunately may be important. They are, however, potentially correctable by further work, especially if and when hybrids can be produced between the species in question. Moreover, the discovery of intermediate forms, if present, can at least indicate whether parallel or convergent evolution has taken place.

One of the 'rules' basic to this type of work is that where there is more than one possible evolutionary pathway to go from one taxon to another, the shortest, most parsimonious route is chosen. A confounding problem may also exist where there is a sharing of inversions among species such that the distribution of

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the inversions among the species cannot be explained by the usual allopatric paradigms. In the *Drosophila repleta* species group, sharing of inversions has been proposed as occurring in the *D. repleta* subgroup and in the *D. mulleri* subgroup (Wasserman 1982, 1992).

The *Drosophila mulleri* species complex of the *mulleri* subgroup was defined by Wasserman (1982) as consisting of 23 cactus-inhabiting species which share and are homozygous for one or more of the following 11 inversions: Xj, Xw, 2c, 2f, 2g, 2h, $2d^2$, $2e^2$, $2s^6$, 3a and 3c. According to Wasserman (1954, 1962, 1982), the ancestor of the *mulleri* complex consisted of a number of semi-isolated cytologically distinct populations which for convenience were referred to as subspecies. Each subspecies gave rise, apparently by means of the usual speciation by geographical isolation, to several extant species. The progeny species of each ancestral subspecies were grouped into six species clusters (Wasserman, 1982). The *mojavensis, longicornis* and *ritae* clusters are essentially North American in distribution; the *buzzatii* and *martensis* clusters are primarily South American; while the *mulleri* cluster is represented throughout the New World.

All of the species of the South American clusters are limited to the New World except for *D. buzzatii* which is now subcosmopolitan (Fontdevila *et al.*, 1981; Barker, 1982). The *martensis* cluster, with four species, is found in the deserts of Colombia and Venezuela, and the nearby islands of the Caribbean (Wasserman & Koepfer, 1979; Ruiz & Fontdevila, 1981); while the *buzzatii* cluster, also with four species, ranges from north eastern Brazil to north western Argentina and Bolivia (Sene *et al.*, 1982, 1988; Ruiz *et al.*, 1982; Wasserman & Richardson, 1987; Fontdevila *et al.*, 1988).

The South American *martensis* and *buzzatii* clusters share three inversions on chromosome 2 which are absent in the other four clusters (Ruiz *et al.*, 1982) and are, thus, phylogenetically quite closely related. The only cytological link between the two South American clusters and the other clusters in the *mulleri* complex is the 3a inversion which was believed to be present in all the species of the four North American clusters except the species *D. arizonae*, and in all the species of the South American *martensis* cluster but not in those of the *buzzatii* cluster (Wasserman, 1982).

We report here the results of a complete re-analysis of the salivary gland chromosomes of the eight species in the *martensis* and *buzzatii* clusters. This re-analysis was fostered by the following observations.

1 Two collecting trips to the West Indies have made available a number of new populations of the two closely related species *D. stalkeri* and *D. richardsoni*, which belong to the *mulleri* subgroup but which were placed in a different species complex, the *stalkeri* complex (Wasserman, 1982). While investigating their salivary gland chromosomes, a striking resemblance was observed by Ruiz between chromosome 2 of *D. stalkeri* and that of *D. buzzatii* uncovering an unexpected possible phylogenetic relationship between the *stalkeri* complex, on the one hand, and the *buzzatii* cluster on the other.

2 Ruiz recently was able to show that 3a was in fact present in *D. arizonae* (Ruiz *et al.*, 1990). This encouraged the re-examination of chromosome 3 in the *martensis* cluster to determine whether the inversion there was indeed 3a.

3 Of considerable importance was the discovery that *D. stalkeri* and *D. richardsoni* hybridize rather easily with some of the species in the *martensis, buzzatii* and *mulleri* clusters (Marin *et al.*, in press). We have made use of this fact to produce hybrids of 11 different interspecific combinations and analyse their salivary gland chromosomes.

Here, we are presenting evidence which demonstrates that: (i) the 3a inversion is not present in the *martensis* cluster. Thus the only remaining link between the two South American clusters and the rest of the *mulleri* complex is broken. The *mulleri* complex is therefore reduced to the *mojavensis, mulleri, longicornis* and *ritae* clusters, while a new complex, the *buzzatii* complex, is established for the South American clusters; (ii) the production of previously unstudied interspecific hybrids demonstrates that the two species of the *stalkeri* complex are related to the *buzzatii* and *martensis* clusters and are, therefore, members of the *buzzatii* complex; (iii) the incorporation of the *stalkeri* complex, as a cluster, into the *buzzatii* complex results in convergent evolution where there are two, nearly equal-length, evolutionary paths from the PRIMITIVE I sequence to the most advanced gene orders; the *stalkeri* cluster is cytologically either the most primitive or the most advanced cluster of the *buzzatii* complex. The simplest phylogenetic tree has the *stalkeri* cluster as the primitive cluster of the complex. This not only results in the 'most parsimonious' phylogenetic tree but also eliminates several examples of alleged sharing of inversions.

Materials and methods

Four stocks of *Drosophila stalkeri* and eight stocks of *D. richardsoni* were cytologically analysed (Table 1). The collection localities cover the entire known geographical range of these two species (see Marin *et al.*, in press, for the geographical position of localities). One strain of *D. stalkeri* from Saint Petersburg (Wasserman, 1962) and one strain of *D. richardsoni*

Table 1 Chromosomal constitution of the 12 stocks of *D. stalkeri* and *D. richardsoni* investigated in this study. Numbers of stocks used in the interspecific crosses are shown in boldface

Drosophila species	Stock number	Locality	Chromosome arrangements				
			Xabc	2mn	3b	4	5
stalkeri	**15801-1451.0**	St. Petersburg, Florida	+	l	+	+	+
	ORV 25	Discovery Bay, Jamaica	+	l	+	+	+
	ORV 28	Little Cayman, Cayman Islands	+	l	+	+	+
	ORV 29	Grand Cayman, Cayman Islands	+	l	+	+	+
richardsoni	**ORV 6**	Fox's Bay, Montserrat	+	w^7y^7	+	+	+
	ORV 7a	Montserrat Airport	+	w^7y^7	+	+	+
	ORV 7b	Montserrat Airport	+	w^7y^7	+	+	+
	ORV 7c	Spanish Point, Montserrat	+	w^7y^7	+	+	+
	ORV 8a	Beef Island, Tortola	+	$w^7y^7p^8$	+	+	+
	OVR 8b	Beef Island, Tortola	+	$w^7y^7p^8$ $w^7y^7q^9$	+	+	+
	ORV 9a	Biras Creek, Virgin Gorda	+	$w^7y^7p^8$	+	+	+
	ORV 9b	Biras Creek, Virgin Gorda	+	$w^7y^7p^8$	+	+	+

called 'from Puerto Rico' (Wasserman, 1982) had been previously studied. The remaining 11 stocks were derived from new material collected in the West Indies by William B. Heed and Marvin Wasserman 3–27 May 1982 (*D. richardsoni*) and 16 November–1 December, 1983 (*D. stalkeri*).

In addition, the salivary gland chromosomes of 13 stocks of the eight described species which make up the *buzzatii* and *martensis* clusters (Table 2) and a strain of *D. mulleri* from Great Inagua were analysed in order to check the inversions fixed in each of the species and to compare them with those fixed in *D. stalkeri* and *D. richardsoni*. These stocks came from the National Drosophila Species Resource Center at Bowling Green or from the stock collection of the Department de Genètica i de Microbiologia, Universitat Autònoma de Barcelona, and have been kept in culture for a number of years.

The monomorphic gene orders of the species *D. repleta*, symbolized as XR, 2R, 3R, 4R, 5R, 6R, had initially been chosen as the standard for the cytotaxonomic study of the *repleta* group (Wasserman, 1954). The salivary gland chromosomes of each species were compared with these sequences and all changes in the gene orders were assumed to be due to 2-break simple paracentric inversions. Each inversion was named in the order in which it was found, the number indicating the chromosome (X, 2, 3, 4, or 5) and the letter specifying the particular inversion (Xa, 3b, $2g^3$, etc.). Each species was then given a cytological formula listing the inversions by which it differed from the species, *D. repleta*. The investigation soon led to the conclusion that the most probable ancestral sequence was not the *repleta* standard but one differing from it by at least six inversions, Xa, Xb, Xc, 2a, 2b, and 3b. The sequence Xabc;2ab;3b;4R;5R was designated as PRIMITIVE I (Wasserman, 1960, 1982).

Parenthetically, while investigating the salivary gland chromosomes of the *buzzatii* complex species, a discrepancy was noted in the proximal region of chromosome 2 (section F6a–H in the map drawn by Wharton, 1942) which should have identical banding pattern in *D. repleta* and many other species, e.g. *D. hydei*. This discrepancy had been already noted by Berendes

Table 2 Chromosomal constitution of the 13 stocks of the *buzzatii* and *martensis* clusters analysed in this study. Numbers of the stocks used in the interspecific crosses are shown in boldface

Drosophila species	Stock number	Locality	Chromosome arrangements				
			Xabc	2abmnz[7]	3b	4	5
*buzzatii**	**BU-C5**	Adeje, Canary Islands	+	+	+	+	g
	BU-2ST	Carboneras, Spain	+	+	+	+	g
serido	**15081-1431.4**	Cafarnaum, Brazil	+	x^7a^8	+	+	+
borborema	**15081-1281.0**	Cafarnaum, Brazil	+	e^8f^8	+	+	+
koepferae	**KO-4**	Vipos, Argentina	+	j^9k^9 $j^9l^9m^9n^9$	k^2	m	w
	KO-9	San Isidro, Bolivia	+	j^9k^9	+	+	+
	KO-11	San Isidro, Bolivia	+	j^9 j^9k^9	+	+	+
martensis	**MA-4**	Guaca, Venezuela	j	f^2	r^2wk	+	d^2
starmeri	**SM-3**	Mal Pais, Curaçao	j jq	e^2e^7 $e^2f^2x^6z^6$	r^2wv	+	d^2
venezolana	**VZ-2**	Piritu, Venezuela	j	e^2e^7	r^2wv	+	d^2
	VZ-10	La Blanquilla, Venezuela	j	e^2e^7	r^2wv	+	d^2
uniseta	UN-2	Guaca, Venezuela	jr	$e^2t^6u^6$	r^2wv	+	d^2
	UN-5	La Boca, Venezuela	jr	$e^2t^6u^6$	r^2wv	+	d^2

*Both stocks were made homokaryotypic for arrangements 2st and 4st in the laboratory.

(1963) who was unable to homologize this region between *D. repleta* and *D. hydei*. Therefore, a detailed (band-by-band) comparison of this region was carried out in several species by A.R. The results of this comparison may be summarized as follows: (i) *D. neorepleta* (stock A30.7 from Madera Canyon, Arizona), a sibling species of *D. repleta*, has in this chromosome region the same sequence as *D. repleta*; (ii) *D. peninsularis* (stock 951.8 from Barahona, Dominican Republic) has a sequence which seems to differ from that of *D. repleta* by a short inversion, $2u^8$, with break-points F6c–G1h; (iii) all other species examined have a sequence which seems to differ from that of *D. peninsularis* by a short inversion, $2t^8$, with break-points G1f–G2f, which partially overlaps $2u^8$. These include *D. hydei* (stock 813.38 from El Salvador), a member of the *hydei* subgroup, and *D. mulleri* (stock from Lake Travis), *D. meridiana rioensis* (stock 403.8 from Huajuapan de Leon, Oaxaca), *D. arizonae* (stock A893 from Navojoa, Sonora), *D. navojoa* (stock A878 from Eldorado, Sinoloa), *D. martensis* (stock from Guaca, Venezuela), and *D. starmeri* (stock from Barquisimeto, Venezuela), all members of the *mulleri* subgroup. The obvious conclusion is that the PRIMITIVE I sequence contains at least two more inversions, $2t^8$ and $2u^8$, than the six originally proposed when compared with the basic *repleta* standard. Nevertheless, these two inversions, overlooked in all past studies, are rather small and do not alter greatly the chromosome's morphology. Even more important, they add nothing to the published phylogenies of the *repleta* group except within the *repleta* subgroup where they have become fixed. Therefore, in order to avoid unnecessarily changing the published information (otherwise the formulae for the standard sequences of most species, and the maps of chromosome 2 would have to be modified)

we will continue to designate Xabc;2ab;3b as the PRIMITIVE I sequence, reserving the use of the $2t^8$ and $2u^8$ inversions for studies within the *repleta* subgroup where these inversions did, in fact, arise.

A number of interspecific crosses involving strains of *D. mulleri* from Great Inagua, *D. stalkeri*, *D. richardsoni* (Table 1), and the eight species in the *buzzatii* and the *martensis* cluster (Table 2) was attempted in small mass cultures of five or 10 pairs. As many larvae as possible from the F_1 of each of the 16 interspecific crosses yielding progeny were dissected and their salivary gland chromosomes studied. Each of the 11 species, except *D. uniseta*, was involved in at least one successful cross with another species. Only the polytene chromosomes of the hybrids of the two crosses *D. starmeri* × *D. venezolana* (Ruiz & Fontdevila, 1981) and *D. buzzatii* × *D. koepferae* (Ruiz *et al.*, 1982) had been previously analysed. They were not repeated here.

Results

The cytogenetic relationships of the 11 species included in this study have been independently investigated in the past: *D. stalkeri* and *D. richardsoni* by Wasserman (1962, 1982); *D. martensis*, *D. starmeri* and *D. uniseta* by Wasserman & Koepfer (1979); *D. venezolana* by Ruiz & Fontdevila (1981); *D. buzzatii* and *D. koepferae* by Ruiz *et al.* (1982); *D. serido* and *D. borborema* by Wasserman & Richardson (1987) and Tosi & Sene (1989); and *D. mulleri* by Wasserman (1962). This is the first time, however, that all 11 species are simultaneously considered and directly compared in a single study.

All the species included in this analysis have similar basic karyotypes consisting of six chromosome pairs: four pairs of equal-length acrocentric autosomes, one pair of dot autosomes, a long acrocentric X and a metacentric, submetacentric or small acrocentric Y (Wasserman, 1982; Wasserman *et al.*, 1983; Baimai *et al.*, 1983; Fontdevila *et al.*, 1988). Hence, the chief interspecific differences in the metaphase chromosomes involve the size and shape of the heterochromatic Y chromosome. The karyotype of *D. serido* is quite variable (Baimai *et al.*, 1983). In some populations of this species the dot is replaced by a submetacentric chromosome due to the acquisition of extra heterochromatin in both arms; in others it appears relatively enlarged due to the addition of heterochromatin in one arm.

Inversion 3a, the link to the mulleri *complex*

The banding of chromosome 3 of *D. martensis* was directly compared with that of *D. mulleri* by A. Ruiz. It was determined that the inversion fixed in *martensis* is not identical to 3a and it is renamed $3r^2$ (break-points are given below). Inspection of chromosome 3 in *D. starmeri*, *D. venezolana* and *D. uniseta* and the analysis of the interspecific hybrids, given below, indicated that the other members of the *martensis* cluster also have $3r^2$, and not 3a. This breaks the only cytological link of the *martensis* cluster and their related species with the *mulleri* complex.

Salivary gland chromosomes of the interspecific hybrids

The chromosomes of the F_1 hybrids produced in 16 different crosses, which amount to 14 interspecific combinations, were observed. A detailed account of the results is given in Appendix A. From these results the following conclusions can be drawn.

The standard chromosome 2 of the *stalkeri* cluster differs from that of the *buzzatii* cluster by only a single inversion, $2z^7$. The standard chromosome 2 of the *buzzatii* cluster differs from that of the *martensis* cluster by only a single inversion, $2e^2$. Thus the relationship among the three clusters is straightforward, being one of the following:

Path (a) *stalkeri* → *buzzatii* → *martensis*;
Path (b) *martensis* → *buzzatii* → *stalkeri*;
Path (c) *stalkeri* ← *buzzatii* → *martensis*.

The inversions, *per se*, do not indicate direction of evolution. However, there is good evidence that PRIMITIVE I is the ancestral sequence of the *repleta* species group. The standard chromosome 2 of the *stalkeri* cluster can be derived from chromosome 2 of PRIMITIVE I by two inversions, 2m and 2n. The standard chromosome 2 of the *martensis* cluster can be derived approximately from chromosome 2 of PRIMITIVE I by three inversions, $2d^2$, $2s^6$, and a new inversion, $2v^8$. The new breakage points for $2s^6$ are F2a and F6a. This is followed by inversion $2d^2$ whose break-points are D3c and F3a and by inversion $2v^8$ whose break-points are D3a and G1-. The latter two inversions overlap and follow inversion $2s^6$. The standard chromosome 2 of the *buzzatii* cluster can be derived from chromosome 2 of PRIMITIVE I only by passing through either *martensis* or *stalkeri*; *buzzatii* is, therefore, eliminated as the ancestral cluster of the *buzzatii* complex.

Thus we see that there are two ways the complex could have evolved from the PRIMITIVE I, either Path (a): PRIMITIVE I to *stalkeri* (2mn) to *buzzatii* ($2mnz^7$) to *martensis* ($2mnz^7e^2$); or Path (b): PRIMITIVE I to *martensis* ($2d^2s^6v^8$) to *buzzatii* ($2d^2s^6v^8e^2$) to *stalkeri* ($2d^2s^6v^8e^2z^7$). The reason for this unusual situation is that the break-points of the inversions are not distri-

buted at random; only four major pieces of chromosome 2 seem to have been moved by the seven possible inversions. In fact, each of the proposed inversions, 2m, 2n, $2d^2$, $2s^6$, $2v^8$, $2z^7$ and $2e^6$, has one of its break-points in the F_2 region, a region consisting of only about six bands. Wasserman (1982), not being aware of the relationship of *stalkeri* to *buzzatii*, had previously chosen Path (b) as the direction of evolution because it was the most parsimonious. However, with the inclusion of the *stalkeri* cluster, Path (a) becomes the most parsimonious, there being only a total of four inversions needed to go from PRIMITIVE I to the *martensis* standard chromosome 2 via Path (a), while five inversions are required to go from PRIMITIVE I to the *stalkeri* standard via Path (b). Further evidence indicating that the *stalkeri* cluster is ancestral is the fact that their X, 3, 4 and 5 chromosomes appear to have not changed from those of PRIMITIVE I, while the *martensis* cluster is homozygous for four new inversions, Xj, 3w, $3r^2$ and $5d^2$. Given the data available at this time, we choose Path (a) as the most probable evolutionary path (see Fig. 1). Following is a description of the salivary gland chromosomes of the species, given the new phylogeny.

Salivary gland chromosomes of the stalkeri *species cluster*

The four stocks of *D. stalkeri* were homozygous for the same arrangements in all chromosomes, i.e. neither polymorphism nor interpopulation differences were found (Table 1). These arrangements differ from PRIMITIVE I by three paracentric inversions, namely 2m, 2n, and 2l (Wasserman, 1962). Inversions 2m and 2n are arranged in tandem and apparently share the middle break-point while inversion 2l follows and overlaps 2m (Fig. 2). The map of the standard chromosome 2 of *D. stalkeri* is shown in Fig. 3a.

The standard sequence of *D. richardsoni* differs from the PRIMITIVE I by four inversions, 2m, 2n, $2w^7$ and $2y^7$ (Table 1). Thus, chromosome 2 of *D. richardsoni* has evolved from the PRIMITIVE I sequence by the fixation of two of the inversions also fixed in *D. stalkeri*, 2m and 2n, plus another two species-specific inversions, $2w^7$ and $2y^7$, one of which is included within the other and shares with it one break-point (Fig. 2). The four stocks of *D. richardsoni* from Montserrat Island were homozygous for the standard chromosome 2 of the species while the four stocks from Tortola and Virgin Gorda contained a polymorphic inversion, $2p^8$ (Fig. 3B). One larva of one stock from Tortola was heterozygous for another inversion on the same chromosome, $2q^8$, included within the $2p^8$ segment and with break-points E5e–D3e.

Salivary gland chromosomes of the buzzatii *species cluster*

The standard sequence of *D. buzzatii* differs from the PRIMITIVE I sequence by four inversions, 5g, 2m, 2n and a new inversion, $2z^7$ (Table 2). This makes the standard chromosome 2 of *D. buzzatii* as $2abmnz^7$ (Fig. 2). The chromosome map for this newer interpretation of the standard arrangement of the *D. buzzatii* chromosome 2 is shown in Fig. 3C and the positions of the common polymorphic inversions are given in Fig. 3C and D.

The *buzzatii* chromosome 2 is the standard for the *buzzatii* cluster. Each of the other three species in the *buzzatii* cluster, *D. koepferae*, *D. serido* and *D. borborema*, has in addition to the *buzzatii* chromosome one further fixed inversion in chromosome 2: $2j^9$ is fixed in *D. koepferae*, $2x^7$ in *D. serido* and $2e^8$ in *D. borborema*. Inversion $2e^8$ is distally located while

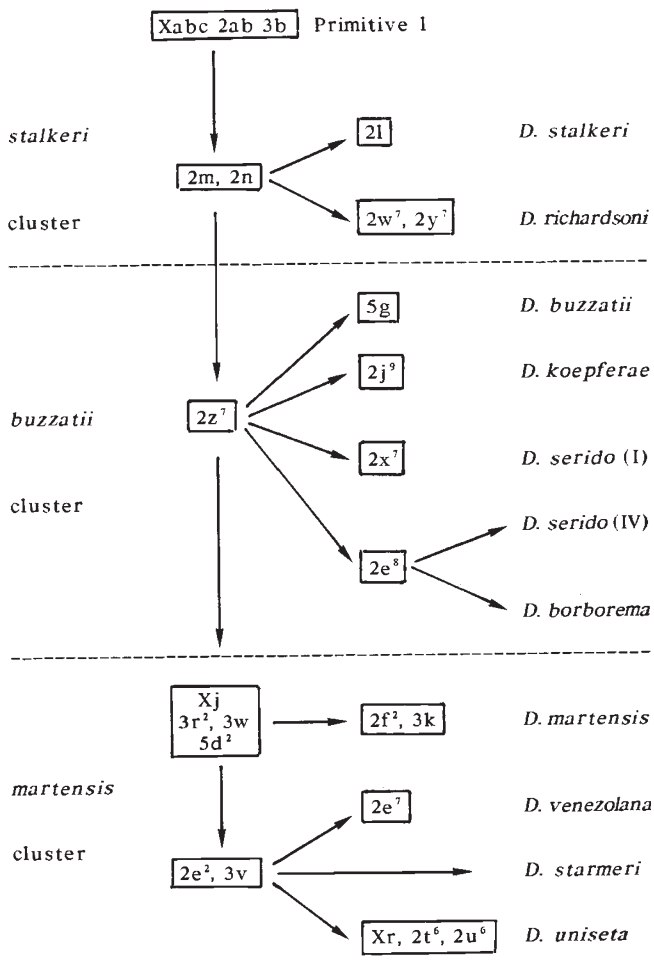


Fig. 1 Chromosomal evolution of the *buzzatii* species complex. Only the paracentric inversions homozygous in each of the species are shown.

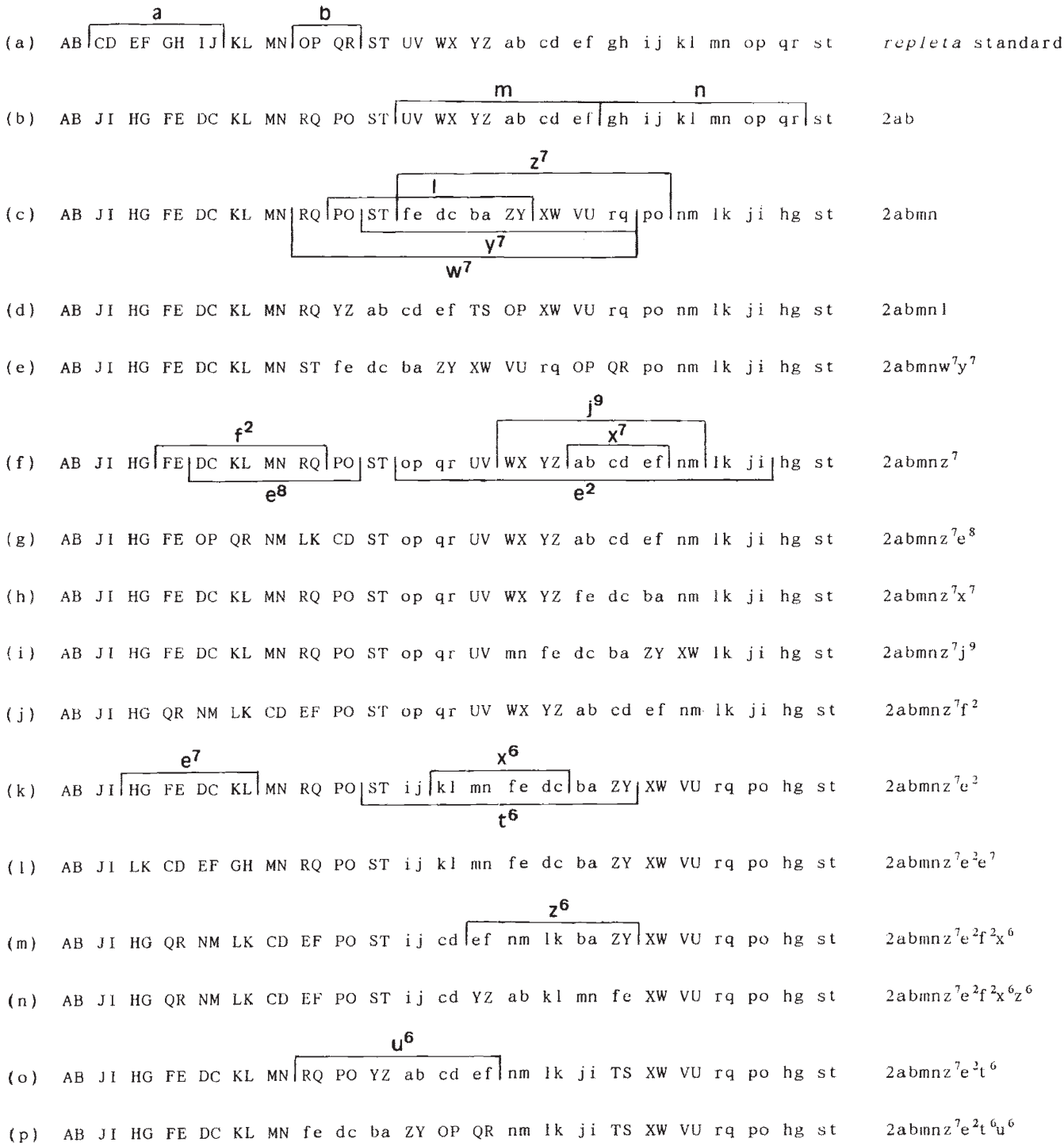


Fig. 2 Evolution of chromosome 2 in the *buzzatii* complex species. Each letter pair represents an unbroken chromosome segment of the *D. repleta* map of Wharton (1942) and the key is given in Appendix B. (a) *D. repleta* sequence showing the positions of 2a and 2b; (b) 2ab, ancestral sequence of the *repleta* species group, showing the break-points of 2m and 2n; (c) 2abmn, ancestral sequence of the *buzzatii* species complex, showing the break-points of 2l (fixed in *D. stalkeri*), $2w^7$ and $2y^7$ (fixed in *D. richardsoni*), and $2z^7$ (fixed in the other species); (d) 2abmnl, standard sequence of *D. stalkeri*; (e) $2abmnw^7y^7$, standard sequence of *D. richardsoni*; (f) $2abmnz^7$, standard sequence of *D. buzzatii* showing the break-points of $2e^8$ (fixed in *D. borborema*), $2x^7$ (fixed in *D. serido*), $2j^9$ (fixed in *D. keopferae*), $2f^2$ (fixed in *D. martensis* and polymorphic in *D. starmeri*), and $2e^2$ (fixed in *D. starmeri*, *D. venezolana* and *D. uniseta*); (g) $2abmnz^7e^8$, standard sequence of *D. borborema*; (h) $2abmnz^7x^7$, standard sequence of *D. serido*; (i) $2abmnz^7j^9$, standard sequence of *D. koepferae*; (j) $2abmnz^7f^2$, standard sequence of *D. martensis*; (k) $2abmnz^7e^2$, standard (hypothetical) sequence of *D. starmeri* with the break-points of $2e^7$ (fixed in *D. venezolana* and polymorphic in *D. starmeri*), $2x^6$ (polymorphic in *D. starmeri*) and $2t^6$ (fixed in *D. uniseta* and polymorphic in *D. starmeri*); (l) $2abmnz^7e^2e^7$, standard sequence of *D. venezolana* which is also found in *D. starmeri*; (m) $2abmnz^7e^2f^2x^6$, sequence ancestral to many of the arrangements present now in *D. starmeri*, with the break-points of $2z^6$ (polymorphic in *D. starmeri*); (n) $2abmnz^7e^2f^2x^6z^6$, chromosome arrangement widespread now in *D. starmeri*; (o) $2abmnz^7e^2t^6$, sequence ancestral to *D. uniseta* with the break-points of $2u^6$ (fixed in *D. uniseta*); (p) $2abmnz^7e^2t^6u^6$, standard arrangement of *D. uniseta*.

inversions $2j^9$ and $2x^7$ occupy a similar proximal region (Fig. 2). The standard sequence of *D. serido* is Xabc;2abmnz^{7}x^7;3b, while that of *D. borborema* is Xabc;2abmnz^{7}e^8;3b (Table 2). The *D. serido* stock analysed in this study was homozygous for one further inversion, $2a^8$, but this inversion is only polymorphic in the species since it coexists with the standard arrangement in other stocks (Wasserman & Richardson, 1987). Likewise, the *D. borborema* stock analysed here was fixed for one further inversion, $2f^8$, which is polymorphic and segregates with the standard arrangement in the two localities investigated thus far (Wasserman & Richardson, 1987). Figure 3E and F shows the break-points of the polymorphic inversions $2a^8$ and $2f^8$.

The standard sequence of *D. koepferae* is Xabc;2abmnz^{7}j^9;3b and was present in one of the stocks from Bolivia (Table 2). In addition, this stock was polymorphic for one inversion in chromosome 2, $2k^9$. A *D. koepferae* stock from Argentina lacked the standard chromosome 2 but was polymorphic for $2k^9$ and three other inversions, $2l^9$, $2m^9$ and $2n^9$ (Fig. 3G and H). In addition, it was homozygous for one inversion in each of the three other major autosomes (Table 2; Ruiz *et al.*, 1982).

Salivary gland chromosomes of the martensis *cluster species*

The standard sequence of *D. martensis* differs from PRIMITIVE I by nine inversions and is Xabcj;2abmnz^{7}f^2;3bkwr^2;4;5d^2 (Table 2). The map of the standard chromosome 2 is shown in Fig. 3I. Inversions 3w and $5d^2$ had been previously overlooked (Wasserman & Koepfer, 1979). The break-points of $3r^2$ and the new inversion 3w are given in Fig. 4 which depicts the evolution of chromosome 3 in the *martensis* cluster. Approximate break-points for $5d^2$ are E1a–F1a.

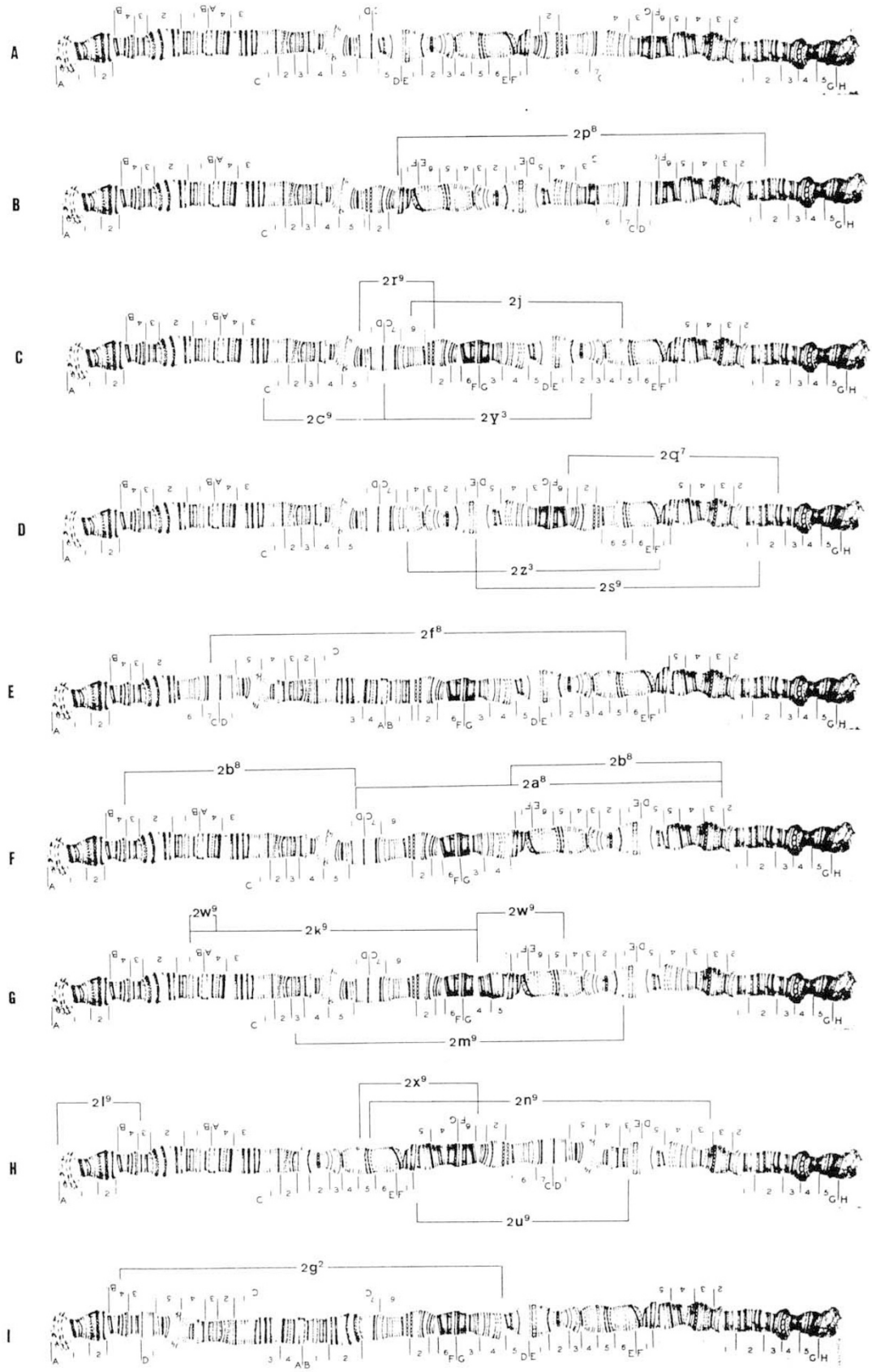
D. starmeri is cytologically the most complex and variable of all the analysed species. Its standard arrangements differ from PRIMITIVE I by nine inversions, seven of which, Xj, 2m, 2n, $2z^7$, $3r^2$, 3w and $5d^2$, are also fixed in *D. martensis* as well as in *D. uniseta* and *D. venezolana* and two, $2e^2$ and 3v, are fixed in the latter two species but absent in *D. martensis* (Fig. 1). Thus, its standard sequence is Xabcj;2abmnz^{7}e^2;3br^{2}wv;4;5d^2 (Table 2). The standard chromosome 2 of *D. starmeri* is hypothetical, i.e. has never been found (Wasserman & Koepfer, 1979; Ruiz & Fontdevila, 1981) nor was it present in the stock studied here. This stock contained two different arrangements derived from the standard chromosome 2, one by the addition of inversion $2e^7$ and the other one by the addition of three inversions, $2f^2x^6z^6$ (Table 2). Inversions $2e^7$ and $2f^2$ are overlapping and mutually exclusive, whereas inversions $2x^6$ and $2z^6$ are also overlapping but independent from $2f^2$ and $2e^7$ (Figs 2 and 3J and K). Thus, in principle recombination may be possible between the two chromosomes. No recombinants have ever been observed, however, in this or other studies. The stock of *D. starmeri* was also polymorphic for inversion Xq. Therefore, its chromosomal constitution was typical of the western race of *D. starmeri* (Wasserman & Koepfer, 1979; Ruiz & Fontdevila, 1981).

The standard sequences of *D. venezolana* and *D. uniseta* differ from PRIMITIVE I by 10 and 12 inversions, respectively (Table 2). That of *D. venezolana* falls entirely within the limits of the chromosomal variation found in *D. starmeri* (Ruiz & Fontdevila, 1981) and can be written as Xabcj; 2abmnz^{7}e^{2}e^7;3br^{2}wv;4;5d^2 (Table 2). *D. uniseta* is homozygous for two species-specific inversions, Xr and $2u^6$, in addition to $2t^6$, which is polymorphic in *D. starmeri* (Fig. 2). Thus, the standard sequence of *D. uniseta* is Xabcjr;2abmnz^{7}e^{2}t^{6}u^6;3br^{2}wv;4;5d^2 (Table 2). Maps of the chromosome 2 standard arrangements of *D. venezolana* and *D. uniseta* are shown in Fig. 3J and L, respectively.

Discussion

Figure 1 shows the chromosomal evolution of the 10 species included in the *buzzatii* species complex and Table 3 summarizes all previous cytological information on these species as well as the new information presented in this paper. This new information modifies our concepts of the relationships and evolution of some of the clusters in this part of the *mulleri* subgroup. The lack of 3a in the *martensis* cluster separates the South American clusters from the rest of the *mulleri* complex. It also eliminates a number of shared, homozygous inversions. The *mulleri* complex can now be defined as consisting of those species that are homozygous for 2g and 3a, while the *buzzatii* complex species are homozygous for 2m and 2n.

The fact that the two complexes are phylogenetically very close to each other is nevertheless attested to by the amount of intercomplex hybridization which can take place in the laboratory. *D. buzzatii* was known to cross with several species of the *mulleri* complex. Patterson & Alexander (1952) reported that *D. buzzatii* females produce F_1 larvae when crossed with *D. wheeleri*; while *D. buzzatii* males produce larvae with *D. arizonae*, sterile F_1 females with *D. wheeleri*, and sterile F_1 males and females with *D. mulleri*. We carried out a series of intercomplex crosses with *D. mulleri* (stock ORV 21 from Great Inagua, the


A
B
2p8
C
2r9
2j
2c9
2y3
D
2q7
2z3
2s9
E
2f8
F
2b8
2a8
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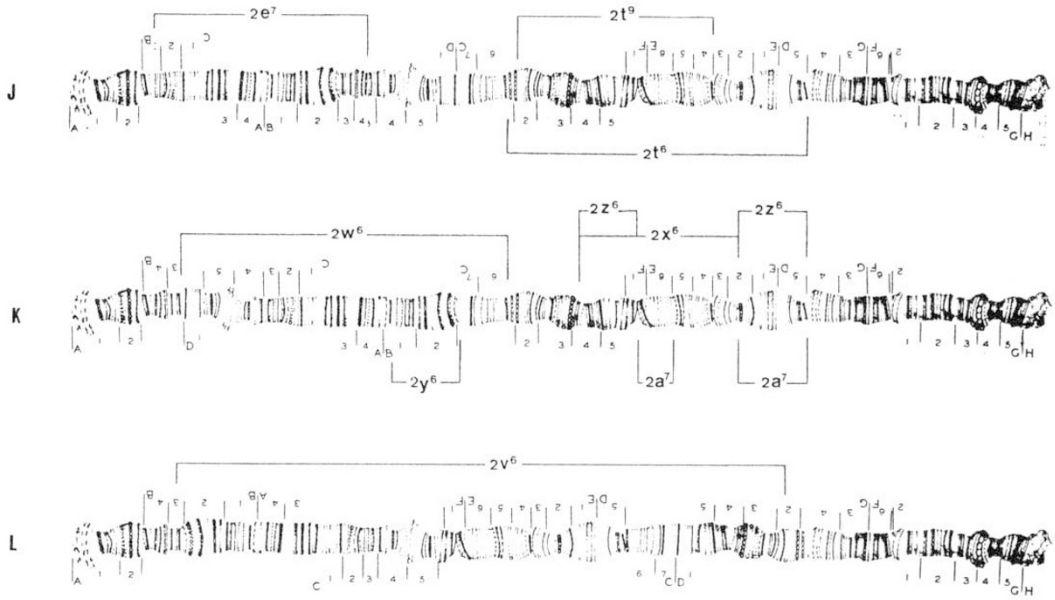

Fig. 3 (this page and opposite) Chromosome 2 maps of the *D. buzzatii* complex species. Each map has been produced rearranging the *D. repleta* map of Wharton (1942) according to the changes shown in Figs 1 and 2. A. 2abmnl, standard sequence of *D. stalkeri*; B. 2abmnw^{7}y^{7}, standard sequence of *D. richardsoni* showing the position of the polymorphic inversion 2p^{8}; C. 2abmnz^{7}, standard sequence of *D. buzzatii* showing the break-points of 2j, 2c^{9}, 2y^{3} and 2r^{9}; D. 2abmnz^{7}j, widespread chromosome arrangement of *D. buzzatii* with the break-points of 2z^{3}, 2q^{7}, 2s^{9}; E. 2abmnz^{7}e^{8}, standard sequence of *D. borborema* with the break-points of 2f^{8}; F. 2abmnz^{7}x^{7}, standard sequence of *D. serido* with the break-points of 2a^{8} and 2b^{8} (that follows and overlaps 2a^{8}); G. 2abmnz^{7}j^{9}, standard sequence of *D. koepferae* with the break-points of 2k^{9}, 2w^{9} (that follows and overlaps 2k^{9}) and 2m^{9}; H. 2abmnz^{7}j^{9}m^{9}, chromosome arrangement of *D. koepferae* showing the break-points of inversions 2l^{9}, 2x^{9}, 2n^{9} and 2u^{9}; I. 2abmnz^{7}f^{2}, standard arrangement of *D. martensis* with the break-points of 2g^{2}; J. 2abmnz^{7}e^{2}e^{7}, standard sequence of *D. venezolana* which is also found in *D. starmeri* showing the positions of 2t^{9} (polymorphic in *D. venezolana*) and 2t^{6} (polymorphic in *D. starmeri*); K. 2abmnz^{7}e^{2}f^{2}, sequence ancestral to some of the arrangements of *D. starmeri* with the break-points of 2w^{6}, 2x^{6}, 2z^{6} (which follows and overlaps 2x^{6}), 2a^{7} (which follows and overlaps 2x^{6} and 2z^{6}) and 2y^{6}; L. 2abmnz^{7}e^{2}f^{2}x^{6}z^{6}, standard sequence of *D. uniseta* with the break-points of 2v^{6}.

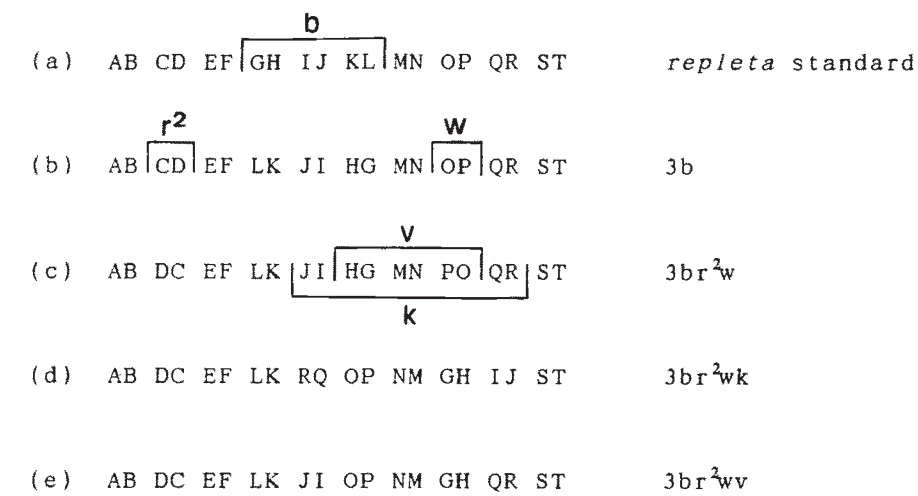

Fig. 4 Evolution of chromosome 3 in the *buzzatii* complex species. Each letter pair represents an unbroken chromosome segment of the *D. repleta* map of Wharton (1942) and the key is given in Appendix B. (a) *D. repleta* sequence showing the position of 3b; (b) 3b, ancestral sequence of the *repleta* species group, showing the break-points of 3r^{2} and 3w; (c) 3br^{2}w, ancestral sequence of the *martensis* cluster species with the break-points of inversions 3k (fixed in *D. martensis*) and 3v (fixed in *D. starmeri, D. venezolana* and *D. uniseta*); (d) 3br^{2}wk, standard sequence of *D. martensis*; (e) 3br^{2}wv, standard sequence of *D. starmeri, D. venezolana* and *D. uniseta*.

Table 3 Chromosomal constitution of the *buzzatii* complex species*

	Fixed inversions					Intraspecific variation	
Drosophila species	Xabc	2abmn	3b	4	5	Polymorphic inversions†	Rare endemics
stalkeri	+	l	+	+	+		
richardsoni	+	w^7y^7	+	+	+	$2p^8$	$2q^8$
buzzatii	+	z^7	+	+	g	2j, $2jz^3$, $2jq^7$, $2jc^9$, $2y^3$, $2r^9$, $2js^9$, 4s	$2e^9f^9$, $2g^9$, $2ji^9$, $2jc^9d^9$, $2jh^9$, $3j^2$, $5c^2$
borborema	+	z^7e^8	+	+	+	$2f^8$	$2f^8g^8$, $2f^8h^8$
serido (I)	+	z^7x^7	+	+	+	$2a^8$, $2a^8b^8$	$2c^8$, $2d^8$
serido (IV)	+	z^7e^8	+	+	+	'2a'	
koepferae	+	z^7j^9	+	+	+	$2k^9$, $2k^9w^9$, $2m^9$, $2m^9n^9$, $2l^9$, $2m^9x^9$, $2l^9m^9n^9$, $2m^9u^9$, "2d", "2e", $3k^2$, 4m, 5w, "5a"	$2m^9v^9$
martensis	j	z^7f^2	r^2wk	+	d^2	$2g^2$	$2g^2o^9$, $2g^2p^9$
starmeri	j	z^7e^2	r^2wv	+	d^2	Xq, Xqs, $2e^7$, $2e^7t^6$, $2f^2x^6z^6$, $2f^2x^6w^6$, $2f^2x^6z^6y^6$, $2f^2x^6z^6a^7$, $2f^2x^6z^6y^6a^7$, $3a^2$, 3z, 3zy, 5q	Xqy, $2e^7t^6q^9$, $2e^7r^7$, $2e^7b^7$, $2f^2x^6z^6y^6a^7c^7$, $3e^2$
venezolana	j	$z^7e^2e^7$	r^2wv	+	d^2	$2t^9$	
uniseta	jr	$z^7e^2t^6u^6$	r^2wv	+	d^2	$2v^6$	

*Sources: Wasserman & Koepfer, 1979; Ruiz & Fontdevila, 1981; Ruiz *et al.*, 1982; Ruiz *et al.*, 1984; Barker *et al.*, 1985; Wasserman & Richardson, 1987; Tosi & Sene, 1989; Fontdevila *et al.*, 1988; unpublished data.
†Only those inversions found in at least two different localities are considered polymorphic.

Bahamas). Females of this *D. mulleri* strain produced third instar larvae when crossed to males of *D. buzzatii*, *D. martensis* and *D. venezolana* (see Appendix A). On the other hand, males of *D. mulleri* produced larvae when crossed to *D. borborema* females but the larvae died in the first or second instar stage and could not be cytologically analysed. All crosses between *D. mulleri* and either *D. stalkeri*, *D. richardsoni*, or *D. uniseta* were unsuccessful. This high level of intercomplex mating is very unusual considering the fact that many species within the *mulleri* complex produce no interspecific hybrid offspring when exposed to members of their own complex. Moreover, the species in the *buzzatii* cluster seem to be more amenable to mating with the *mulleri* complex species than are the species in the *D. stalkeri* cluster which are supposed to be more primitive and therefore more closely related to the *mulleri* complex than are the *buzzatii* cluster species. A possible explanation for this paradox is that *D. stalkeri* and *D. richardsoni* are sympatric with several species of the *mulleri* complex (Wasserman & Wasserman, 1992) and thus there has been the opportunity for character displacement in sexual isolation.

It is not easy to fit the cytological phylogeny depicted in Fig. 1 with the geographical distribution of the species. The three clusters of the *buzzatii* complex are allopatric. The cytologically most primitive, *stalkeri*, is limited to the Caribbean Islands and Florida; the most advanced, *martensis*, has an intermediate distribution and is found in Venezuela and

Colombia; while the *buzzatii* cluster is known from Brazil, Argentina, and Bolivia. This situation is somewhat similar to that found by Heed & Russell (1971) in the *cardini* group, where populations and species with a central distribution are cytologically derived while those in the margins are more conservative. A possible scenario for the evolution of the *buzzatii* complex is that the ancestor lived in the general region now occupied by the *martensis* cluster. There was an early invasion of the Caribbean Islands by 2mn forms which evolved into the *stalkeri* cluster. A later invasion of Brazil by $2mnz^7$ forms led to the *buzzatii* cluster whereas the central area continued to evolve and is now the *martensis* cluster. As was suggested in the previously published phylogenies (Wasserman, 1982), the species *D. martensis* still occupies an intermediate step between the *buzzatii* cluster and the rest of the *martensis* cluster.

Our ideas as to the evolution of the species within the clusters have not changed. *D. richardsoni* and *D. stalkeri* appear to be allopatric species, both having arisen from the 2mn ancestral migrant. The cytological evolution of the *martensis* cluster is essentially as was depicted by Wasserman & Koepfer (1979). The only difference is that the direction of evolution from the species *D. martensis* to *D. buzzatii* should be reversed. The ancestor of the *martensis* cluster appears to have been a highly polymorphic form which has evolved into the present-day *D. starmeri*. The other species split off from this ancestor, each fixing its own inversions, many of which have remained polymorphic in *D. starmeri*. This scenario suffers from the fact that the derived species are not peripherally located to the central polymorphic species. The four species are virtually completely sympatric and there is no present-day evidence of allopatry in the *martensis* cluster.

Our knowledge of the *buzzatii* cluster is fragmentary. The way *D. serido* has been treated in Fig. 1 and Table 3 deserves a comment. *D. serido* is a superspecies which consists of many semi-isolated populations ranging from the Caatinga in northeastern Brazil to the Monte in northwestern Argentina (Sene *et al.*, 1982, 1988; Ruiz *et al.*, 1982; Fontdevila *et al.*, 1988). From the point of view of the salivary gland chromosomes, the populations hitherto studied have been classified by Tosi & Sene (1989) into four chromosomal types. Type I is found in the Brazilian Caatinga and include the type locality of the species as well as the localities investigated by Wasserman & Richardson (1987) and that studied here. Type III includes the populations of the Monte and western Chaco in Argentina. These populations, first analysed by Ruiz *et al.* (1982) who called them Argentinian *D. serido*, were later described as a separate species, under the name of *D. koepferae*, by Fontdevila *et al.* (1988). The populations found in Bolivia also belong to *D. koepferae* as they are fully fertile with those in northwestern Argentina. Type II include the localities in the eastern Chaco in Argentina. According to Tosi & Sene (1989) these populations are fixed for the $2j^9$ inversion, are polymorphic for one inversion on chromosome 5, which they call '5a', and are heterozygous on chromosome 2 for two other inversions, '2d' and '2e'. We have included these populations in *D. koepferae* based on the observation that they are fixed for the $2j^9$ inversion. However, this inclusion must be considered only tentative until more critical data are obtained. Finally, type IV include the populations in central and western Brazil. Tosi & Sene (1989) state that these populations are not fixed for the $2x^7$ inversion, but are homozygous for the $2e^8$ inversion which is fixed in *D. borborema*, and polymorphic for another inversion which they call '2a'. Since these populations also exhibit a different aedeagus morphology and show reproductive isolation from most other *D. serido* populations, Tosi & Sene (1989) suggest that they probably represent a separate, and yet undescribed, species.

A total of 84 inversions has occurred during the evolution of the *buzzatii* species complex (Table 4). Nineteen are homozygous, fixed, inversions. None of them is shared in the sense discussed above. Three inversions are polymorphic in one species but homozygous, fixed, in a sister species. $2e^7$ is fixed in *D. venezolana*, $2f^2$ is fixed in *D. martensis* and $2t^6$ is fixed in *D. uniseta*; all three inversions are polymorphic in *D. starmeri*. The number of fixed inversions per species varies between 3 in *D. stalkeri* and 12 in *D. uniseta*, with an average of 6.1.

Table 4 Number of paracentric inversions homozygous or heterozygous in the evolution of the *D. buzzatii* complex

	Chromosome					
Inversions	X	2	3	4	5	Total
Homozygous intercomplex	0	2	0	0	0	2
Homozygous intercluster	1	1	2	0	1	5
Homozygous intracluster	0	2	1	0	0	3
Homozygous interspecific	1	6	1	0	1	9
Homozygous and heterozygous*	0	3	0	0	0	3
Intraspecific polymorphism†	2	29	4	2	3	40
Rare endemics	1	18	2	0	1	22
Total	5	61	10	2	6	84

*Inversions which are polymorphic in one species but homozygous, i.e. fixed, in a sister species.
†Only those inversions present in at least two different localities are considered polymorphic.

Sixty-two inversions are intraspecific variation. No shared heterozygous inversions, i.e. those polymorphic in both of two daughter species, were found. The intraspecific variation has been classified tentatively into two categories: rare endemics and polymorphic inversions. By rare endemics we mean those inversions which have been found in a single locality only, almost always with a low frequency. Polymorphic inversions are those found in at least two different localities. This classification is necessary as the number of inversions described in a given species is a function of the amount of effort invested in collecting and sampling. For instance, in *D. melanogaster* over 320 different inversions have been found in nature, although only seven are at all widespread or common (Ashburner, 1989). Any comparison among species, therefore, must take into account the different efforts devoted to different species. The number of polymorphic inversions per species varies between zero in *D. stalkeri* and 14 in *D. starmeri* with an average of 3.9. The number of rare endemics per species varies between zero in *D. stalkeri*, *D. venezolana* and *D. uniseta*, and eight in *D. buzzatii* (the species most extensively sampled in the complex) with an average of 2. These figures are relatively high for the *repleta* group with a mean value of only 2.1 polymorphic inversions per species (Wasserman, 1992b). Why the *buzzatii* complex is more polymorphic than the other complexes or subgroups, and why some species are more variable than others remain open questions.

Acknowledgements

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Appendix A: salivary gland chromosomes of the interspecific hybrids

The chromosomes of the F_1 hybrids produced in 16 different crosses, which amount to 14 interspecific combinations, were observed. The crosses are listed below with the species which provided the female parent first.

1 *D. stalkeri* (1451.0)×*D. buzzatii* (BU-2ST). A single female larva dissected. Good general pairing except proximal 1/7 of chromosome X and proximal end of chromosomes 3 and 5. Chromosomes X, 3 and 4 are homosequential. Chromosome 2 shows regions A–C5, D5–F1 and F5–H paired, regions C7–G4 of the *D. buzzatii* chromosome and F6–D2 of the *D. stalkeri* chromosome unpaired for they have no counterpart in the same place of the homologous chromosome, giving rise sometimes to two small single-chromosome loops (as expected for two overlapping inversions, $2l/z^7$). Chromosome 5 has a single loop in proximal one-half ($5g/+$).

2 *D. stalkeri* (1451.0)×*D. borborema* (1281.0). Twenty-eight larvae dissected. Chromosome X short and thick in males, unpaired in proximal 1/4 in females. Chromosomes X, 3, 4 and 5 are homosequential except for minor band differences in proximal end of chromosomes X, 3 and 5. Chromosome 2 shows regions A–B4 and F5–H paired, rest of chromosome unpaired often giving rise to a complex multi-inversion loop (compatible with $2l/z^7e^8f^8$).

3 *D. stalkeri* (ORV 28)×*D. borborema* (1281.0). Eight larvae dissected. Chromosomes as in cross **2**.

4 *D. stalkeri* (ORV 28)×*D. venezolana* (VZ–10). Two larvae dissected. Chromosome X has a small single loop in proximal one-half ($Xj/+$). Chromosome 2 shows one distal single loop ($2e^7/+$) and a complex multi-inversion loop in proximal one-half (compatible with $2l/z^7e^2$). Chromosome 3 has one distal single loop ($3r^2/+$) and a double loop in proximal one-half ($3wv/+$). Chromosome 4 is homosequential. Chromosome 5 has a small single loop in proximal one-half ($5d^2/+$).

5 *D. richardsoni* (ORV 8b)×*D. buzzatii* (BU–C5). Two male larvae dissected. Chromosome 2 shows regions A–C5 and G3–H often paired, rest of chromosome unpaired implying a complex multi-inversion loop (compatible with $2w^7y^7p^8/z^7$). Chromosomes 3 and 4 are homosequential. Chromosome 5 has a single loop in proximal one-half ($5g/+$).

6 *D. richardsoni* (ORV 6)×*D. koepferae* (KO–4). Two male larvae dissected. Chromosome 2 shows regions A–B2 and F3–H paired, rest of chromosome unpaired implying a complex multi-inversion loop (compatible with $2w^7y^7/j^9k^9$). Chromosome 3 has single loop in proximal one-half ($3k^2/+$). Chromosome 4 has a small single loop in its proximal quarter ($4m/+$). Chromosome 5 has a small single loop in proximal one-half ($5w/+$).

7 *D. richardsoni* (ORV 6)×*D. venezolana* (VZ–10). Eighteen larvae dissected. Chromosome X shows a small single loop in proximal one-half ($Xj/+$). Chromosome 2 shows a distal single loop ($2e^7/+$) and a complex multi-inversion loop involving proximal 2/3 (compatible with $2w^7y^7/z^7e^2$). Chromosome 3 has a distal single loop ($3r^2/+$) and a double loop in proximal one-half ($3wv/+$). Chromosome 4 is homosequential. Chromosome 5 has a small single loop in proximal one-half ($5d^2/+$).

8 *D. richardsoni* (ORV 8b)×*D. venezolana* (VZ–2). Six larvae dissected. Chromosome 2 has a distal single

loop ($2e^7/+$) and a complex multi-inversion loop in proximal 2/3 (compatible with $2w^7y^7p^8/z^7e^2$). All other chromosomes as in cross **7**.

9 *D. martensis* (MA-4) × *D. richardsoni* (ORV 6). Two larvae dissected. Chromosome X has a single loop in proximal one-half (Xj/+). Chromosome 2 shows a single loop in distal one-half ($2f^2/+$) and a complex multi-inversion loop in proximal one-half (compatible with $2w^7y^7/z^7$). Chromosome 3 shows a distal single loop ($3r^2/+$) and a double loop in proximal one-half (3wv/+). Chromosome 4 is homosequential. Chromosome 5 has a small single loop in proximal one-half ($5d^2/+$).

10 *D. serido* (1431.4) × *D. koepferae* (KO-9). Eight larvae dissected. Excellent general pairing except in proximal end and distal tip of all chromosomes. Chromosomes X, 3, 4 and 5 homosequential. Chromosome 2 shows regions A–B1 and G1–H paired, rest of chromosome would seem at first sight to have a double loop (corresponding to only two overlapping inversions); however, when the banding pattern is compared in detail, it is realized that two chromosome segments (D4 and F5), although paired, are not truly homologous implying four inversions of difference between the two chromosomes (as expected $2x^7a^8/j^9k^9$).

11 *D. koepferae* (KO-9) × *D. serido* (1431.4). Twelve larvae dissected. Chromosomes as in cross **10**.

12 *D. borborema* (1281.0) × *D. venezolana* (VZ-10). Thirteen larvae dissected. Distal one-half of chromosome X always paired, proximal one-half often unpaired showing sometimes what seems to be a single loop (Xj/+). Chromosome 2 is usually totally unpaired implying a complex multi-inversion loop (compatible with $2e^2e^7/e^8f^8$). Chromosome 3 has a distal single loop ($3r^2/+$) and a double loop in proximal one-half (3wv/+). Chromosome 4 is homosequential. Chromosome 5 has a small single loop in proximal one-half ($5d^2/+$).

13 *D. koepferae* (KO-9) × *D. starmeri* (SM-3). Twenty-four larvae dissected. Chromosome X has a single loop in proximal one-half (Xj/+). Chromosome 2 shows a complex multi-inversion loop (which corresponds to $2j^9k^9/e^2e^7$ in some larvae and to $2j^9k^9/f^2x^6z^6$ in others). Chromosome 3 has one distal single loop ($3r^2/+$) and a double loop in proximal one-half (3wv/+). Chromosome 4 is homosequential. Chromosome 5 has a small single loop in proximal one-half ($5d^2/+$).

14 *D. mulleri* (MU-6) × *D. buzzatii* (BU-2ST). A single female larvae dissected. Total asynapsis in all chromosomes. Homologous chromosomes are completely separated in most nuclei and only touch each other, when they do, in two or three points along the chromosomes' length. Inversion loops are not formed and synapsis is absent even in those chromosomes which are expected to be homosequential (e.g. chromosome 4). Hybrids are not useful for assessing the inversion differences between the parental species.

15 *D. mulleri* (MU-6) × *D. martensis* (MA-4). Two male larvae dissected. Chromosome X short and very thick. Total asynapsis in all autosomes. See comments to cross **14**.

16 *D. mulleri* (MU-6) × *D. venezolana* (VZ-10). Five larvae dissected. Chromosome X short and very thick in males. Total asynapsis in all chromosomes. See comments to cross **14**.

Appendix B: key to the chromosome segments of Figs 2 and 4

A key to the segments of the salivary gland chromosomes shown in Figs 2 and 4 is provided. Each pair of letters in the two figures represents an unbroken segment of the *D. repleta* chromosome maps (Wharton, 1942).

Chromosome 2 (Fig. 2)

AB:	A→A3a	QR:	C7e→D1g	gh:	F2a→F3a
CD:	A3a→B2a	ST:	D1g→D3d	ij:	F3a→F4a
EF:	B2a→B3a	UV:	D3d→D4a	kl:	F4a→F4d
GH:	B3a→B4e	WX:	D4a→D5a	mn:	F4d→F6a
IJ:	B4e→C1a	YZ:	D5a→D5c	op:	F6a→G1a
KL:	C1a→C3f	ab:	D5c→E2c	qr:	G1a→G1g
MN:	C3f→C6a	cd:	E2c→F1c	st:	G1g→H
OP:	C6a→C7e	ef:	F1c→F2a		

Chromosome 3 (Fig. 4)

AB:	A→B1c	IJ:	D4b→D5a	OP:	E4a→F4c
CD:	B1c→C5d	KL:	D5a→E1a	QR:	F4c→G1h
EF:	C5d→D3d	MN:	E1a→E4a	ST:	G1h→H
GH:	D3d→D4b				