Chromosome Phylogeny of *Drosophila pachea* and Related Species

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SPECIES groups in the genus *Drosophila* are established on the basis of morphological similarities and differences. For a review of this natural group see Stone[14]. The classification of species into species groups has been reinforced, with few exceptions, by within-group comparisons of the banding sequences of the giant polytene chromosomes found in the larval salivary glands. Sturtevant and Dobzhansky[16] were first to recognize that from a series of overlapping inversions it is possible to reconstruct the evolutionary history of the chromosome. Phylogenetic relationships may also be derived from independently segregrated inversions by arranging the species according to chromosomal banding similarities[21]. Wasserman[22] discussed the above methods and concluded that the resulting phylogenies have a very high degree of accuracy.

Six major groups in the subgenus *Drosophila* have been analyzed using one or both of the above methods: 1) *virilis* species group[15]; 2) *repleta* species group[21]; 3) *melanica* species group[13]; 4) *cardini* species group[4]; 5) picture-wing group of Hawaiian *Drosophila*[2]; and 6) *mesophragmatica* species group[1]. In the *tripunctata*[6] and the *guarani* species group[7] the number of fixed inversion differences between species were too large for complete analysis.

We have examined four morphologically distinct desert-inhabiting species of *Drosophila* and have found them to share the same banding pattern in each of the seven polytene salivary arms except for one intraspecific and four interspecific inversions. Three of the species were originally assigned to different subgenera; the fourth is an undescribed species. An abstract of portions of this work has been published elsewhere[19].

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Materials and Methods

Collection records

D. acanthoptera, *D. nannoptera*, and species w were collected in the more arid habitats of southern Mexico, whereas *D. pachea* is found closely associated with the Sonoran desert in northwestern Mexico and Baja California.

D. nannoptera (H381.6, Acatlan, Puebla, Mexico) was collected by Dr. Marvin Wasserman in 1958–1959. This species was previously collected by Drs. Marshall R. Wheeler[23] and F. A. Cowan (297 specimens) 60 miles south of Oaxaca, Oaxaca, Mexico near the valley of the Rio Tehuantepec on September 6, 1947. That culture (1808.1) was lost and therefore not available for examination.

D. acanthoptera (1808.21, Oaxaca, Oaxaca, Mexico) was collected by Drs. Marshall R. Wheeler[23] and F. A. Cowan on September 4, 1947 about 60 miles south of Oaxaca, Oaxaca, Mexico near the valley of the Rio Tehuantepec. Two females and one male were collected.

Species w (113.14, Tehuantepec, Oaxaca, Mexico) was collected by Dr. Marvin Wasserman during August 14–16, 1966 about 3 klm north of Tehuantepec. The culture was started with a total of 31 individuals (personal communication).

D. pachea, from 27 localities in Sonora and Baja California, Mexico, was obtained during 1962–1969 from flies reared from rotting senita cacti, *Lophocereus schottii* var. *schottii* (Engelmann) Britton and Rose, *L. schottii tenuis* Lindsay[9], and *L. gatesii* M. E. Jones (see also Heed *et al.*[5]). Generally more than 100 adults originated each culture. Collection localities, rearing records, and the chromosomal polymorphism of *D. pachea* will be published in detail[18].

Only one culture was available for each of the other three species (see above). These cultures were started from adults netted over traps baited with fermenting bananas. The cultures are maintained on standard laboratory food medium, and in the case of *D. pachea* each vial is supplemented with a small cube of autoclaved senita cactus[3].

Taxonomic history

D. acanthoptera and *D. nannoptera* were dissimilar enough taxonomically so that the subgenus

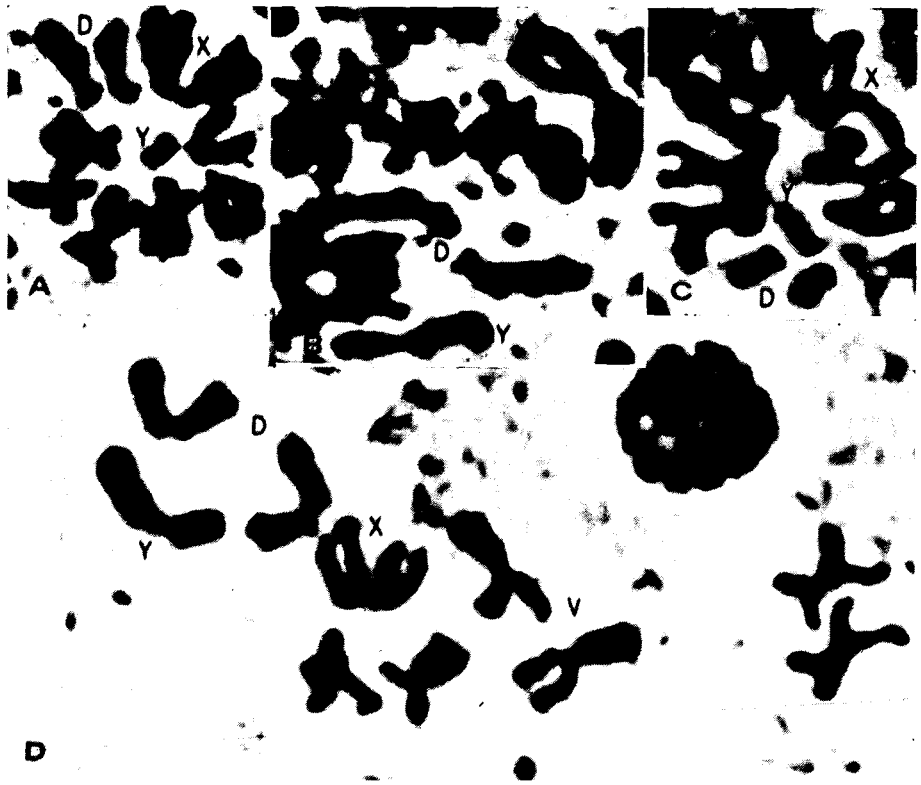

FIGURE 1 — Photomicrographs of *D. pachea* and related species. *A*—*D. pachea*; *B*—*D. acanthoptera*; *C*—species w; *D*—*D. nannoptera*; Symbols are: D—dot and added heterochromatin; X—X chromosome, Y—Y chromosome, and V—chromosome 3, which has added heterochromatin in *D. nannoptera*.

Sordophila was specifically erected for the first species and the second species was assigned to a monotypic species group in the subgenus *Sophophora*[23]. Throckmorton[17], in constructing a phylogeny based on the adult internal morphology, transferred *D. nannoptera* to the subgenus *Drosophila* to conform with the results he obtained from examining a large number of *Drosophila* species. *D. pachea* had been assigned to the subgenus *Drosophila* with the desert dwelling *repleta* species group[11], but Throckmorton[3] found *D. pachea* more similar to *D. nannoptera*. Throckmorton has not examined *D. acanthoptera*.

Hybridization

Interspecific hybridization was attempted by mass mating per vial, 20–50 adult virgin females (aged 1–10 days) with an equal number of males of a different species. Culture A156 of *D. pachea* was used in all crosses involving this species. All possible crosses were made, many crosses were repeated, and at various intervals during the crosses the seminal receptacle and the spermatheca were dissected from a number of females and inspected for sperm.

Cytology

Salivary gland polytene chromosomes[12] and somatic metaphases[8] from giant neuroblast cells of the larval hindbrain were prepared using standard lactic-acetic-orcein methods. The banding sequence in culture A156 of *D. pachea* was chosen as the standard (+) banding sequence of the four species. All cytological comparisons were made against this sequence. All elements except for number 7 are monomorphic (contain no heterozygous inversions). Element 7 is polymorphic for a second sequence. The one found in the other three species will be considered as standard (+) for that arm. A cytological map was drawn of the standard banding sequence with the aid of a Wild drawing tube (Wild Heerbrugg Instruments, Inc.).

Photomicrographs were taken of the standard banding sequence and were visually compared, band-by-band, to squash preparations and photomicrographs of the other three species to detect any inversions that had become homozygous (fixed) between the species (interspecific inversions). The culture(s) were examined for intraspecific inversions using two methods. Cultures from 16 localities representing the entire distribution of *D. pachea* and one culture for each of the other species were examined for the presence of heterozygous inversions in all 7 salivary arms. Cultures of *D. pachea* from 16 localities were each mass mated (approximately 10 pairs) to the standard culture (A156) and at least one female larva was examined for the presence of heterozygous inversions. This latter method detects fixed differences between cultures.

Results

Hybridization

A total of 15 interspecific crosses were made with the four species and no hybrids were found (Table I). Moreover, none of the 114 females examined had

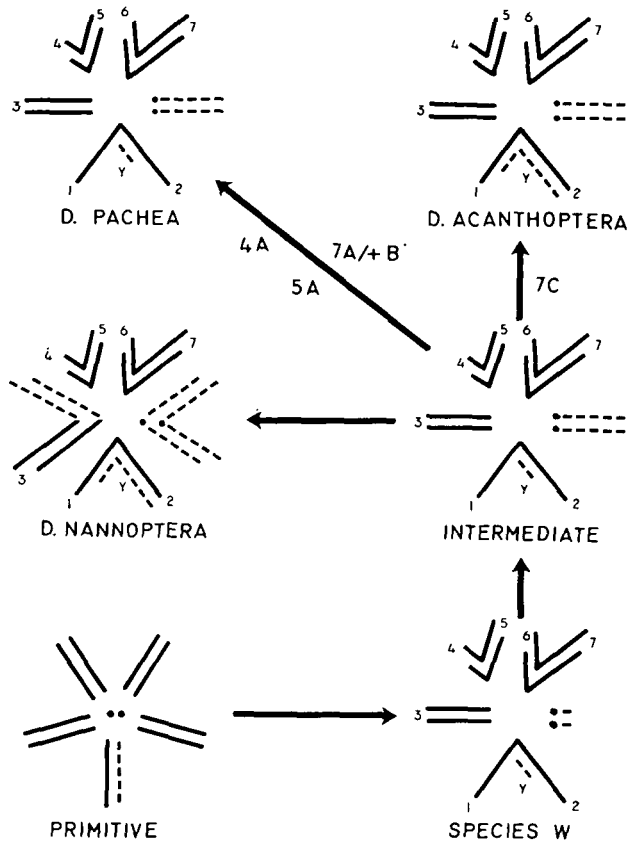

FIGURE 2—The karyotype phylogeny of *D. pachea* and related sepcies. Each karyotype is diagrammatic; solid lines indicate euchromatic and dashed lines indicate heterochromatic arms. The numbers adjacent to the metaphase chromosomes refer to the salivary element(s) that is most probably homologous to that particular chromosome. The assignment of homologues was based on four observations: 1) salivary elements 1 and 2 stain noticeably lighter in males of this group; 2) in squash preparations of salivary nuclei elements 4-5 and 6-7 are found consistently associated together at their bases; 3) there is a noticeable correlation between the relative sizes of salivary element(s) and indicated metaphase chromosome; 4) the heterochromatic and the euchromatic parts of the metaphase chromosomes can be distinguished in Figure 1 as heavily stained regions and separate chromatids, respectively. Inversion differences between species are represented by the large symbols (4A, etc.) beside the arrows.

been inseminated. This indicates that strong sexual isolation exists between each species.

Karyotypes

Photomicrographs of the somatic metaphase chromosomes (Figure 1) and the diagrammatic representations of the four karyotypes (Figure 2) show that the four species differ only by additions of heterochromatin to the autosomes or Y chromosome. The karyotype of species w (Figure 1*C*) consists of one large V (=X), one short rod (=Y), one pair of rods, one pair of short heterochromatic rods with a small satellite, and two pairs of J's, one smaller than the other. *D. pachea* (Figure 1*A*) and *D. acanthoptera* (Figure 1*B*) are both karyotypically identical to species w except for the Y and heterochromatic rod. *D. acanthoptera* and *D. pachea* both have a larger pair of heterochromatic rods with a satellite. The satellite is noticeably larger in *D. pachea* than in *D. acanthoptera* or species w. The satellites are not indicated in Figure 2. The Y chromosome is a short rod in *D. pachea* and a large J in *D. acanthoptera*. The karyotype of *D. nannoptera* (Figure 1*D*) is composed of one large V (=X), one large J (=Y), two pairs of large V's (one totally heterochromatic with a secondary constriction, the other V having one heterochromatic arm), and two pairs of J's, one smaller than the other. The hypothetical primitive dot chromosome has progressively increased in amounts of heterochromatin from species w (short pair of rods with a satellite), *D. acanthoptera* (pair of rods with a small satellite), *D. pachea* (pair of rods with a satellite), to *D. nannoptera* (pair of large V's with a secondary constriction).

The karyotype of *D. acanthoptera* agrees with that previously published by Ward[20] except that we found one chromosome pair as a small J, whereas he found it to be a small V. He had also examined *D. nannoptera* but his description differs from ours by finding the Y to be a large rod and by no mention of the secondary constriction on the large V pair.

The reorganization of the primitive karyotype of five rods and a dot to form the one now found in species w consisted of at least one X-autosomal centric fusion, two pericentric inversions in separate rod chromosomes[10], and a small addition of heterochromatin to the dots. These changes would account for the seven arms and one dot (eight polytene elements) now found in the salivary nuclei of species w. Inasmuch as the other three species also have seven arms and a dot (Figures 4–9), additions of

Table 1. The number and sex of *D. pachea*, *D. acanthoptera*, *D. nannoptera*, and species w used to prepare the interspecific matings and the resultant inseminated females/total number examined

Species crosses			Number crossed		Number of females inseminated/ total examined
Female	×	Male	♀	♂	
pachea		*acanthoptera*	31	33	0/5
			34	50	
		nannoptera	50	50	0/9
		species w	50	50	0/20
acanthoptera		*pachea*	69	89	
		nannoptera	18	37	0/5
			47	28	
		species w	18	30	
nannoptera		*pachea*	50	50	0/20
		acanthoptera	40	25	
			30	44	
		species w	50	50	0/19
species w		*pachea*	50	50	0/20
		acanthoptera	81	30	0/10
		nannoptera	50	50	0/6

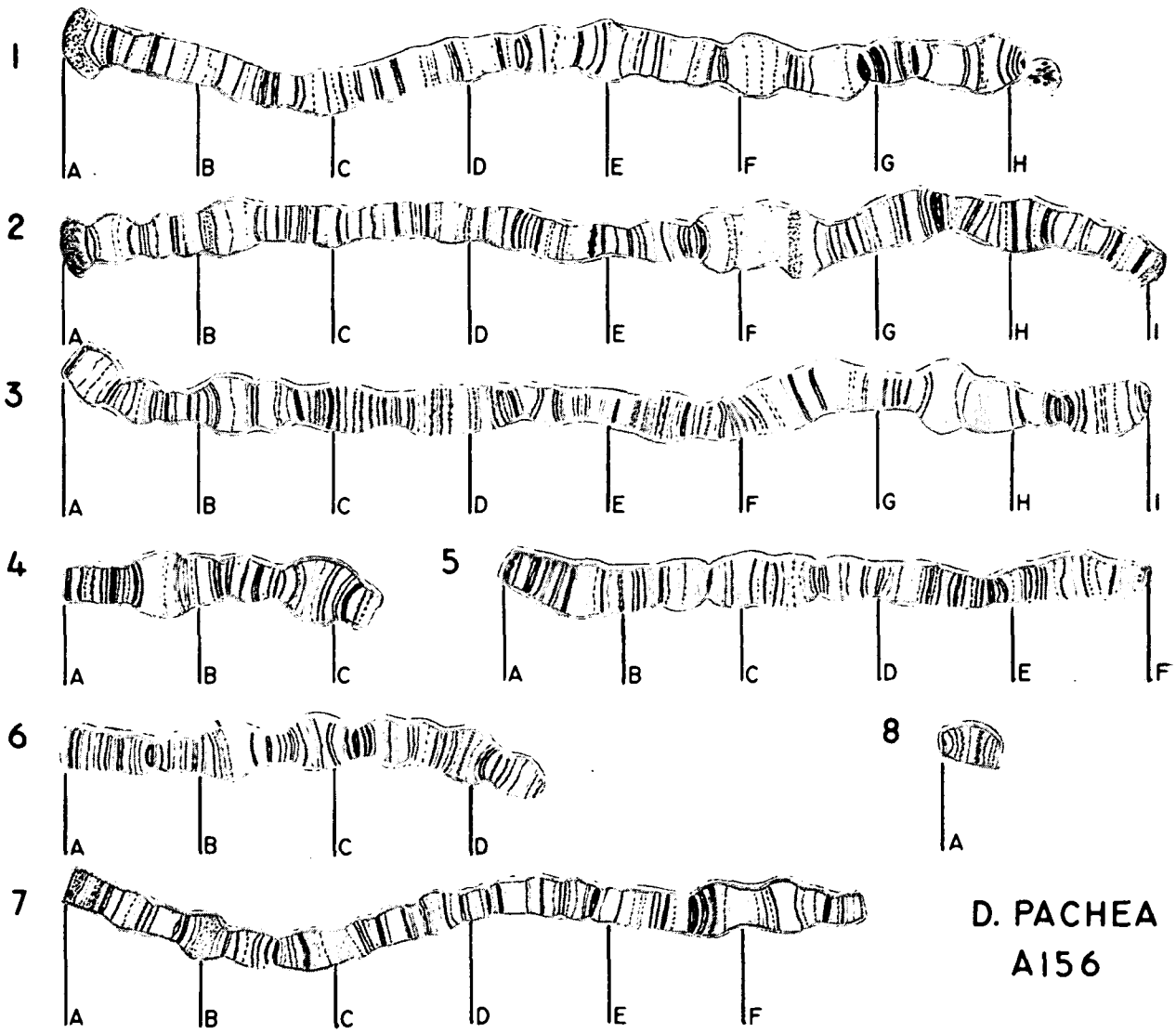

FIGURE 3—A cytological map of the eight salivary gland elements of *D. pachea* (A156) showing the standard banding sequence. The elements are numbered 1 through 8 and are each divided into equal, lettered segments beginning with the distal and ending at the centromeric end of the elements.

heterochromatin to the existing karotype chromosomes must account for the additional changes observed. Specifically, the rods with a satellite found in *D. pachea* and *D. acanthoptera* result from an addition of heterochromatin to the pair of short rods in species w (Figure 2). *D. nannoptera* can be derived from the proposed intermediate with additions of heterochromatin to both pairs of rods and the Y-chromosome. It is significant that no known member of this group has been found with the primitive karyotype of five rods and a dot, which is present in most other species groups thus far analyzed cytologically.

Salivary chromosomes

Figure 3 is a cytological map drawn to show the standard banding sequence of the eight salivary gland elements of *D. pachea* (A156). Photomicrographs of the banding sequences and the inversions of the salivary elements 1 through 8 are shown in Figures 4–9. Each inversion found was labeled using a number corresponding to the salivary element involved and a letter naming the inversion in the order of discovery. Elements 1, 2, 3, and 6 each have the same banding sequence (the standard) in all four species (Figures 4, 5, 6, and 8). By convention, inversions 4A, 5A, and 7B of *D. pachea* are considered as part of the standard banding sequence for elements 4, 5, and 7 respectively (Figures 7 and 9). *D. nannoptera* and species w have identical sequences on all eight elements and therefore are homosequential species[2]. Strikingly, they have the

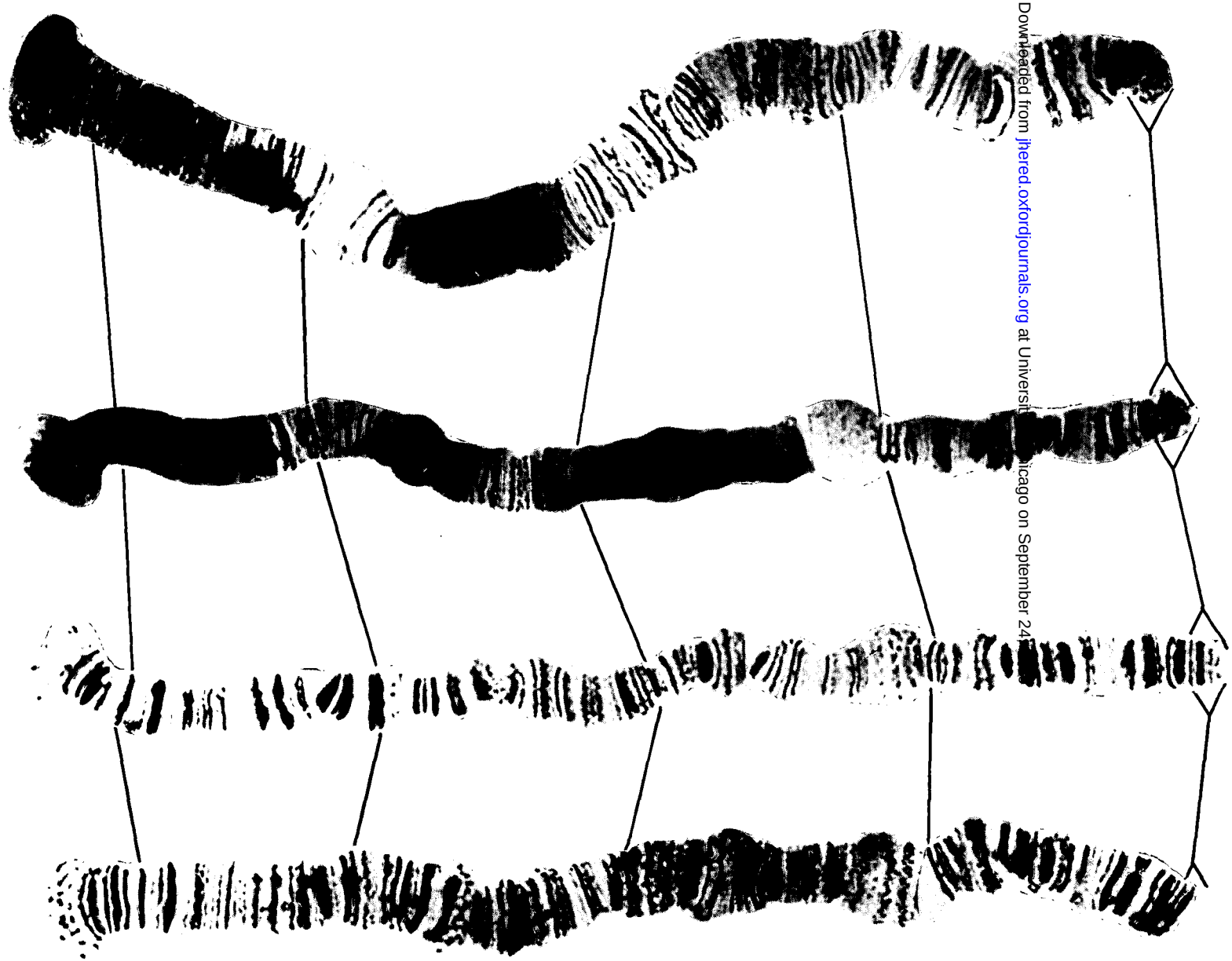

FIGURE 4—Composite photomicrographs of the salivary element 1 of *D. pachea* and related species. The distal ends of the elements are all placed at the left margin. From top to bottom the species are: *D. pachea*, *D. acanthoptera*, *D. nannoptera*, and species w. Vertical lines between the elements indicate homologous bands. From species w to *D. pachea* there has been either a progressive increase or a different organization of material within the brackets at the base. This material appears to be heterochromatin in the upper two species.

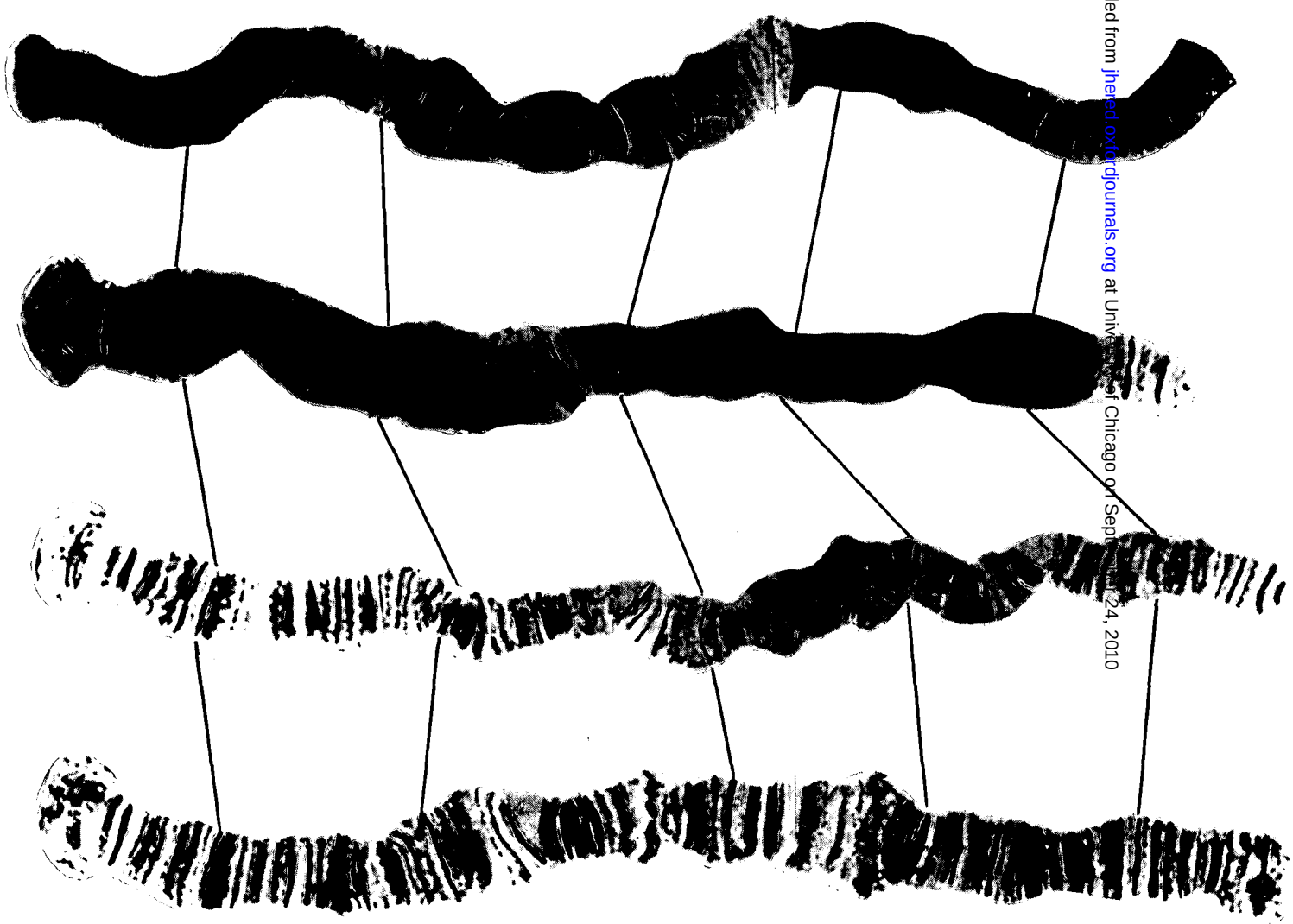

FIGURE 5—Composite photomicrographs of the salivary element 2 of *D. pachea* and related species. The distal ends of the elements are all placed at the left margin. From top to bottom the species are: *D. pachea*, *D. acanthoptera*, *D. nannoptera*, and species w. Vertical lines between the elements indicate homologous bands.

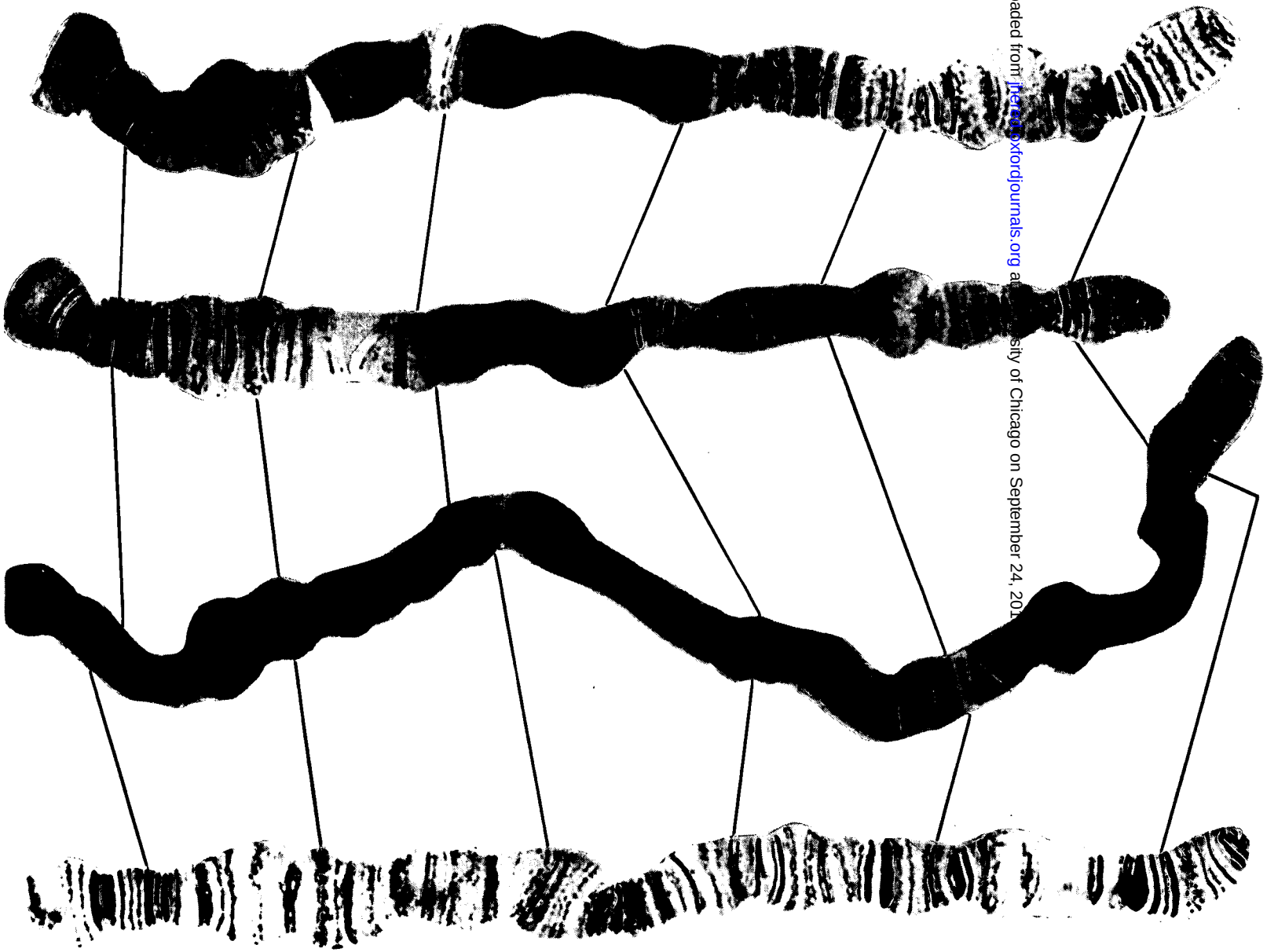

FIGURE 6—Composite photomicrographs of the salivary element 3 of *D. pachea* and related species. The distal ends of the elements are all placed at the left margin. From top to bottom the species are: *D. pachea*, *D. acanthoptera*, *D. nannoptera*, and species w. Vertical lines between the elements indicate homologous bands.

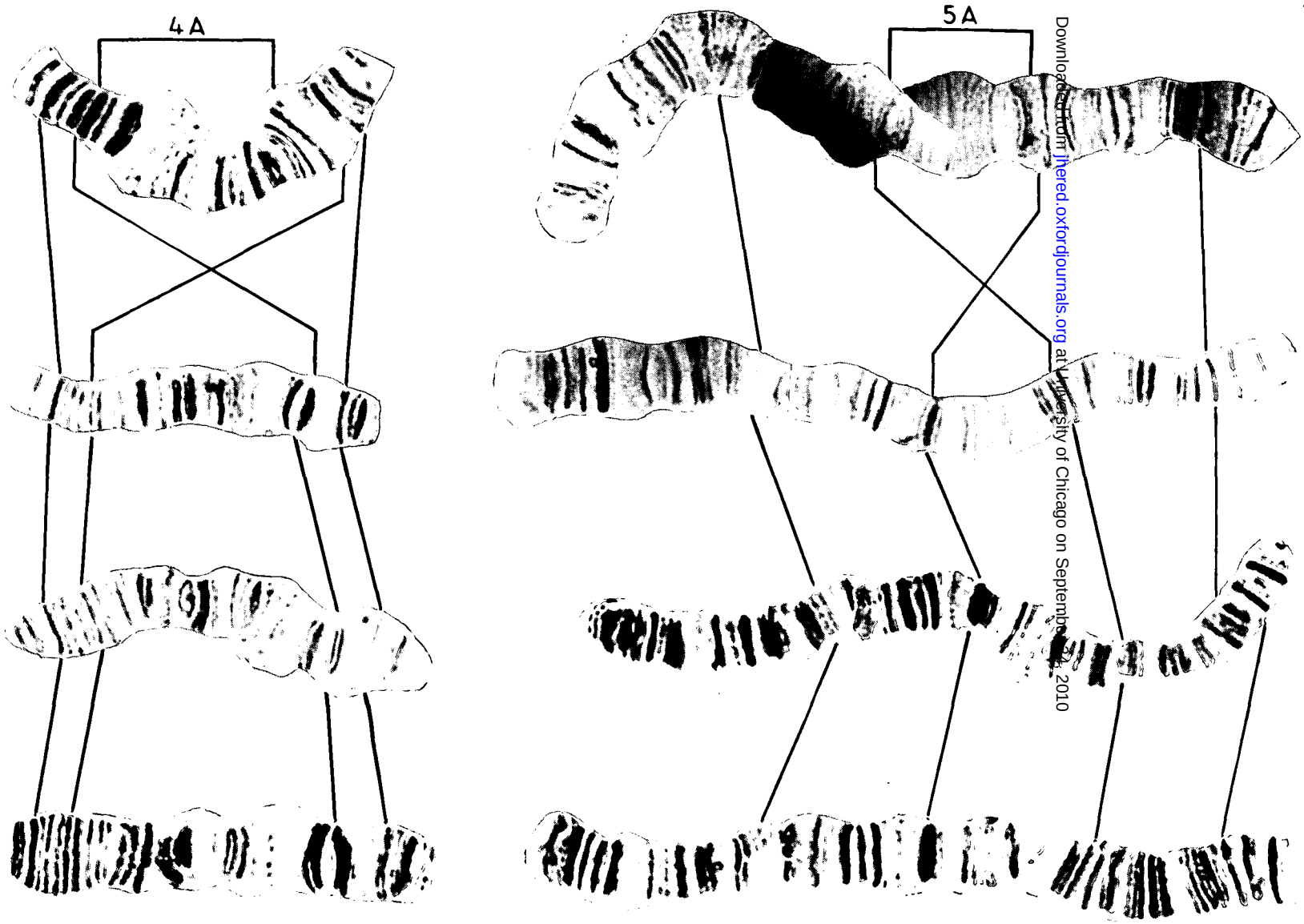

FIGURE 7—Composite photomicrographs of the salivary elements 4 (left) and 5 (right) of *D. pachea* and related species. The distal ends of the elements are all placed toward the left margin. From top to bottom the species are: *D. pachea*, *D. acanthoptera*, *D. nannoptera* and species w. Vertical lines between the elements indicate homologous bands. The bracket above the chromosome indicates the extent of each inversion. The standard sequence for elements 4 and 5 contain inversions 4A and 5A in *D. pachea*.

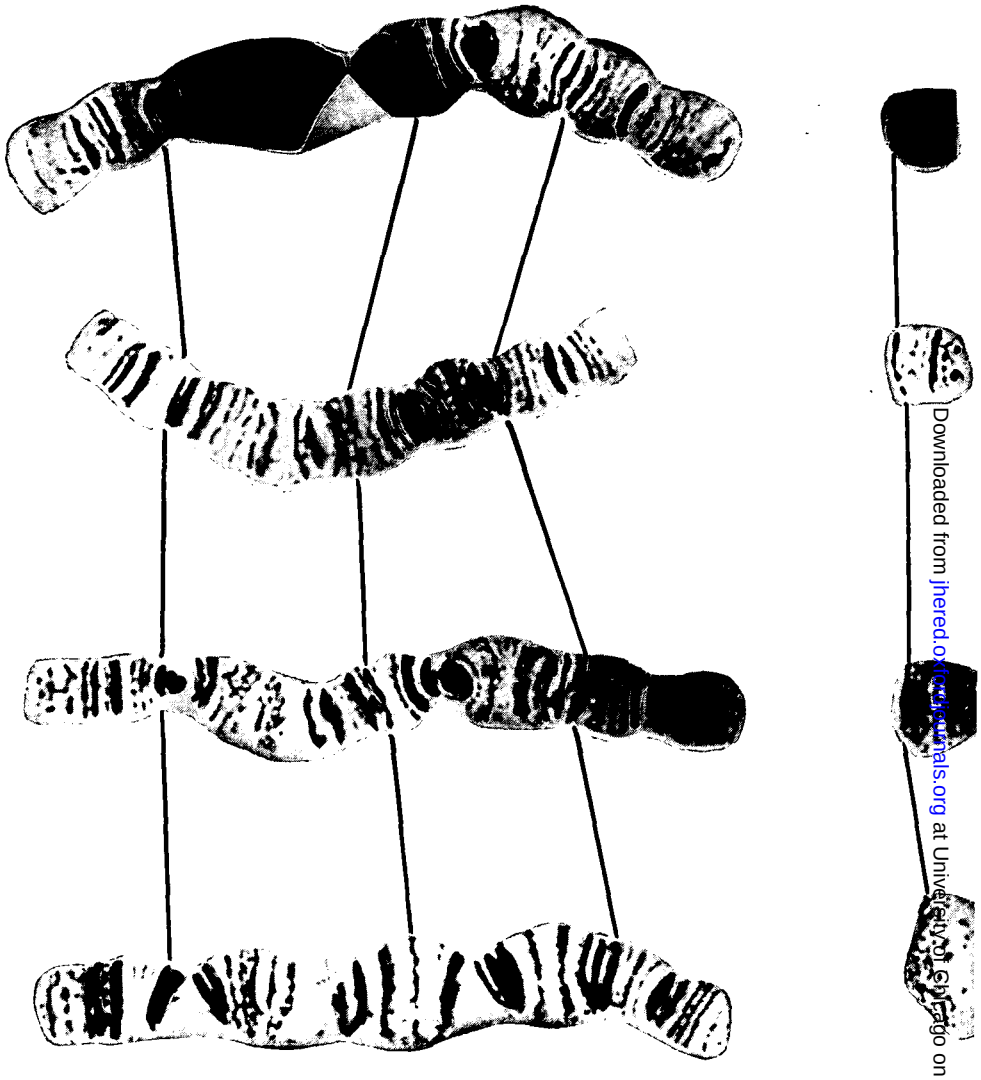

FIGURE 8 — Composite photomicrographs of the salivary elements 6 (left) and 8 (right) of *D. pachea* and related species. The distal ends of the elements are all placed toward the left margin. From top to bottom the species are: *D. pachea*, *D. acanthoptera*, *D. nannoptera* and species w. Vertical lines between the elements indicate homologous bands. The banding in the dot (8) element of species w is unorganized, especially toward the basal end. In *D. nannoptera* the dot characteristically has attached to the base a large round staining body.

most dissimilar metaphase karyotypes of the four species. *D. acanthoptera* differs from the above two species by inversion 7C while *D. pachea* has fixed three other inversions (4A, 5A, and 7B) and carries 7A heterozygous (Figure 2).

In the salivary gland nuclei of *Drosophila* species the centromeric heterochromatin of each element or arm fuses together to form a chromocenter. This facilitates the counting and delimiting of each salivary arm since their bases are normally all held together by the chromocenter with the arms radiating from the chromocenter like the spokes in a wheel. When the arms do break free, enough heterochromatin usually comes with it to indicate the basal end. Squash preparations of the salivary gland nuclei lack a true chromocenter in *D. pachea*, *D. acanthoptera*, *D. nannoptera* and species w. This prevents the counting of salivary arms by the above methods. However, in most of our squash preparations the seven arms separate from one another in a consistent pattern. Certain arms are repeatedly found attached in pairs. Specifically 1 and 2, 4 and 5, and 6 and 7 normally remain attached. However, arm 1, for example, can be found sometimes attached to arm 3 or any other arm. The region of attachment is always the same for each arm and in the majority of cases appears as a partial gap or narrow portion of the two arms. Except in the case of 1, there is no heterochromatin to indicate the basal end of the arms.

As mentioned above, the four species each have seven salivary arms and a dot. There are three long, two medium, and two short salivary arms, plus a dot. Ward[20] found only five long arms, one short arm, and a dot chromosome in *D. nannoptera*. This culture (1808.1) was not available for examination. Even so, the observation was probably erroneous. It is likely that what he counted as a long arm was either the 4 and 5 or the 6 and 7 arms attached in pairs by a cryptic amount of heterochromatin at their bases.

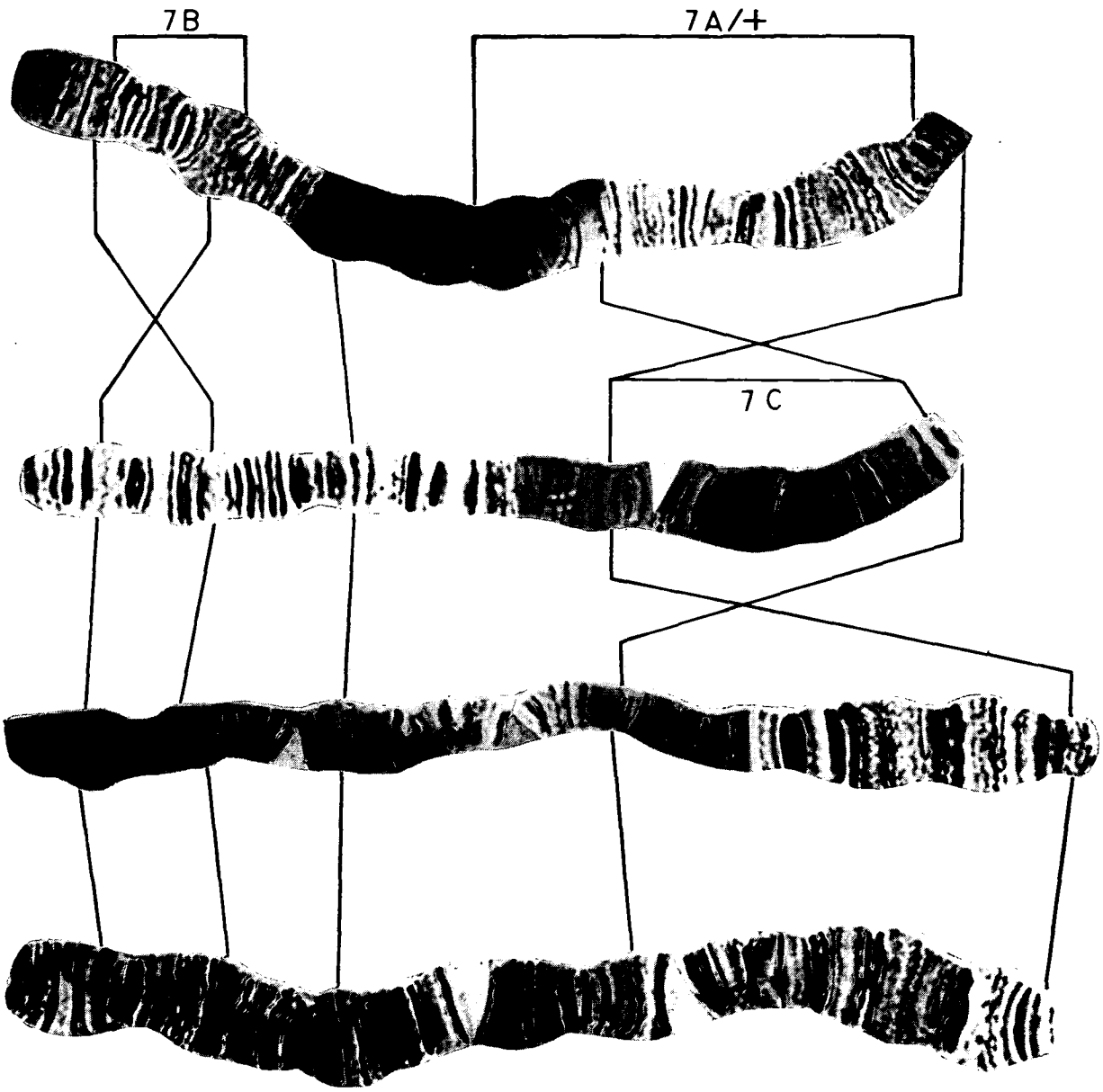

FIGURE 9—Composite photomicrographs of the salivary element 7 of *D. pachea* and related species. The distal ends of the elements are all placed at the left margin. From top to bottom the species are: *D. pachea*, *D. acanthoptera*, *D. nannoptera*, and species w. Vertical lines between the elements indicate homologous bands. The bracket above the chromosome indicates the extent of each inversion. The standard sequence of *D. pachea* contains inversion 7B fixed and carries 7A heterozygous. *D. acanthoptera* has fixed inversion 7C.

Discussion

In the great majority of cases the technique of salivary gland chromosome analysis has confirmed unambiguously the classifications based upon morphological similarity. However, the technique has also exposed relationships where little or none were expected (divergent evolution) and also has shown cases of little relationship where it was expected on morphological grounds (parallel or convergent evolution).

In the former case, Carson[2] has found that the large idiomyia flies in the Hawaiian Islands, formerly classified as the genus *Idiomyia*, have chromosomal banding patterns very similar to the picture-wing group of *Drosophila*. Phenotypically the idiomyia flies differ from all other Hawaiian flies by their very large size and by an extra posterior crossvein in the wing. The genus *Idiomyia* was placed in synonomy.

In the latter case, Wasserman[21] discovered that *D. penninsularis*, formerly a member of the *mulleri*

subgroup of *Drosophila* of the *repleta* group, was not chromosomally related to members of this subgroup and it was removed even though morphologically it is very similar to members of the *mulleri* subgroup. The more recent discovery by Kastritsis[7] that the *guarani* species group of *Drosophila* may be treated more conveniently as two species groups is an example of parallel evolution in morphology.

The present report is another discovery of an unsuspected relationship between distinct species. However, we reserve the decision concerning their formal taxonomic relationships until each species has been reworked morphologically. In any event, the four species represent an element unrelated to the *repleta* species group that has become adapted to arid land conditions. The four species also exhibit a low amount of inversion polymorphism, as far as tested, which is similar to the cytological characteristics of the *repleta* group[21]. Conditions which may be responsible for restricting the success of inversions in the four species will be discussed in a later paper[18].

Summary

Examination of polytene salivary chromosomes has revealed an unsuspected phylogenetic relationship between four species of *Drosophila* where previously none was expected based on morphological grounds. *D. nannoptera* (Puebla, Mexico) and an undescribed species w (Oaxaca, Mexico) have identical banding sequences in each salivary chromosome (=homosequential). *D. acanthoptera* (Oaxaca, Mexico) differs from the above species by one fixed inversion. *D. pachea* (Sonora and Baja California, Mexico) has fixed three additional inversions and carries one more heterozygous. The three described species, which were originally assigned to three different subgenera and two monotypic species groups, differ by only four fixed inversions. The somatic metaphase karyotypes differ by additions of heterochromatin. Attempts at interspecific hybridization have failed because of sexual isolation. The taxonomic relationship of the species must remain uncertain until each has been re-examined morphologically. Although apparently unrelated to the *repleta* species group, the four species have also become adapted to arid land conditions and exhibit the same low level of inversion polymorphism characteristic of the *repleta* species group.

ADDENDUM: Since this manuscript was submitted, additional collections made by the senior author during the summer of 1970 in Puebla and Oaxaca, Mexico, strengthens our previous cytological conclusions. *D. acanthoptera* and species w (one locality each), and *D. nannoptera* (three localities) each exhibit their previously known banding sequences. *D. nannoptera* (two localities) was found to be segregating for the standard sequence and a new inversion (7D) in element 7. The inversion is immediately adjacent to the proximal break of 7B and slightly overlaps the distal break of 7A. All three species of *Drosophila* were found associated with and reared from various genera of giant columnar cacti.

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