Chromosomal Polymorphism in *Drosophila birchii*

V. BAIMAI

USE of salivary gland chromosomes in *Drosophila* has made possible extensive studies of chromosomal variations in natural populations. Comparisons of naturally occurring chromosomal inversions have been utilized as an excellent tool for determination of phylogenetic relationships within and between *Drosophila* species groups. Instances of this type of investigation are the works of Dobzhansky and his associates in the *D. obscura* group[12], the *D. [illegible]pleta* group[24], the *D. virilis* group[22], and the *D. melanica* group[21]. Chromosomal polymorphism has been extensively studied in a number of temperate species, e.g., *D. pseudoobscura*, *D. robusta* and *D. willistoni*. The relevance of chromosomal rearrangements to environmental conditions seems clear as the outcome of such studies in the genetics of natural populations[8].

Cytogenetic studies in *Drosophila* species from the Australasian region show an interesting situation regarding speciation, as was recently demonstrated by Mather[17, 18] in *D. rubida*, and by Angus[1] in *D. tetrachaeta*. *Drosophila birchii*, a sibling species of *D. serrata* and *D. dominicana*, is a member of the *melanogaster* species group of the subgenus *Sophophora* and has been found only in this area. The existence of sexual isolation between geographically isolated populations has been reported by Dobzhansky and Mather[14], Ayala[2], and Baimai[4]. Moreover, cytological investigations of metaphase plate figures in this species have revealed a remarkable karyotype variation[3]. This is a potentially interesting situation. The giant salivary chromosomes are easily workable and have been shown by quantitatively analyzing a [illegible] chromosomal inversion polymorphisms. These features have led to the selection of *D. birchii* for further research in hope of obtaining data on its chromosomal polymorphism and phylogenetic relationships.

Materials and Methods

The materials employed in this study were derived from fresh collections made by the author and his colleagues. The collections of wild flies were made from fermenting bananas exposed in rain forests at Cairns (Northern Queensland); Daru, Port Moresby, Popondetta, Bulolo, Wewak (in the territory of Papua-New Guinea); and at Rabaul (New Britain).

Wild-caught males of *D. birchii* can be readily identified on the basis of their characteristic genitalia, whereas the females are indistinguishable by visible morphology from those of their sibling species. Usually wild-caught females are already inseminated prior to capture and retain sperm for up to eight weeks or so. By the time all the sperm seem to have been exhausted, the females appear to have less sexual activity and low fecundity. Thus in this study, the cytological analyses were based on both wild-caught males and F_1 male progenies of females already inseminated in natural populations. Each of the male flies was allowed to mate with an individual female of standard gene arrangement in a pair mating tube; tubes were stored at the optimal temperature (65° ± 1° F). Larvae were fed with thick yeast suspension to give optimal food to ensure large, cytologically favorable salivary gland chromosomes. The salivary chromosomes of F_1 hybrid larvae were then examined for inversions. Seven or more F_1 larvae including at least one female were required to ensure a 98 percent probability of testing both haploid chromosome sets from each parent.

The giant salivary chromosomes were studied in lacto-aceto-orcein squash preparations of salivary gland cells after pretreatment with acetic alcohol and aceto-carmine solutions. The method adopted [illegible] by Kastritsis[15]. The chromosomal inversions were recorded photographically on 35 mm Kodak Panatomic-X under oil immersion with a green filter.

Results

The chromosomes of D. birchii

The ganglion metaphase complement normally consists of two pairs of V's, one pair of dots and one pair of sex chromosomes of various types. The salivary gland cell contains five long arms, and one short one. These euchromatic arms are easily recognized on the basis of their characteristic tips.

Dr. Baimai is a lecturer in the Department of Biology, Mahidol University, Bangkok, Thailand. The work was supervised by Dr. Wharton B. Mather, head of the Genetics Laboratory, Department of Zoology, University of Queensland, and arises out of a thesis for which the Ph.D. degree was awarded by the University of Queensland in May, 1969.

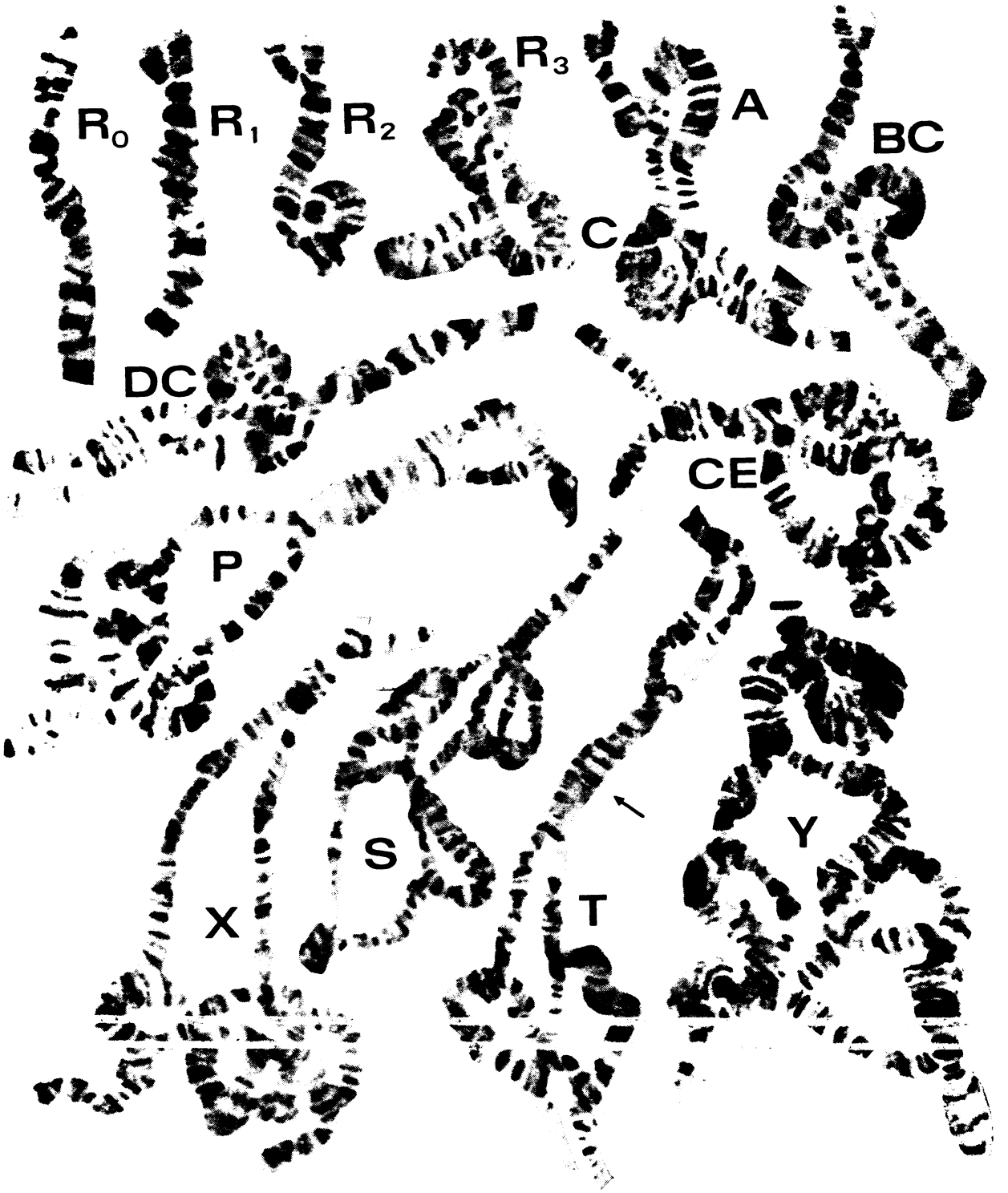


FIGURE 1—Heterozygous simple and complex inversions in chromosome 1 of *Drosophila birchii*. The arrow indicates an extra interstitial band in the T complex.

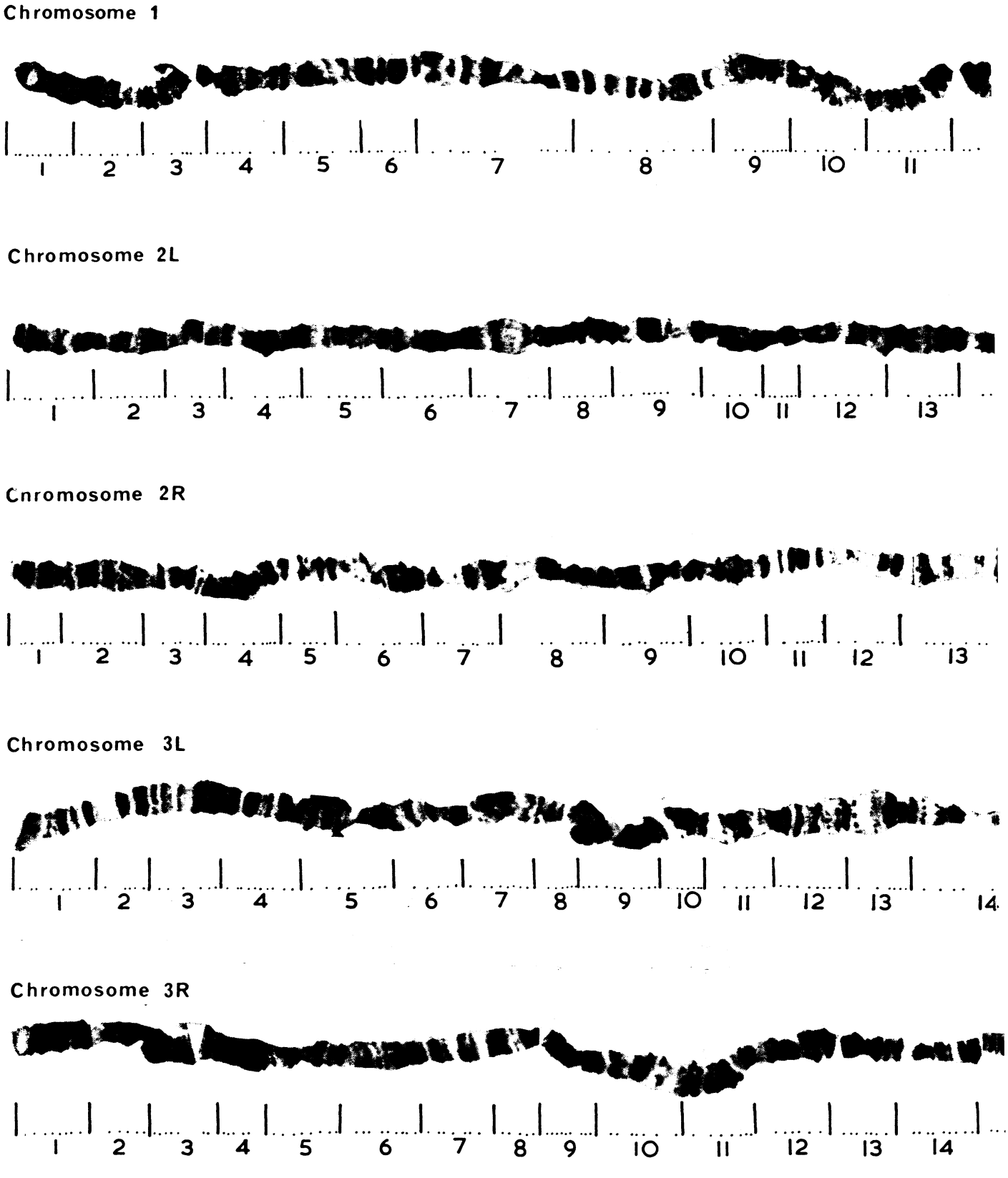
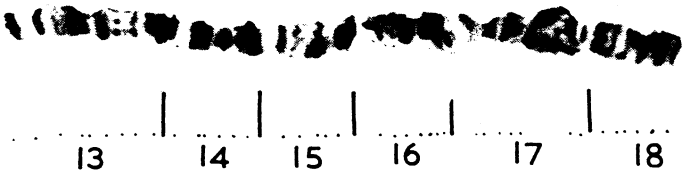


FIGURE 2—*D. birchii* giant chromosome photographic map.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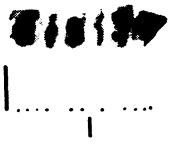
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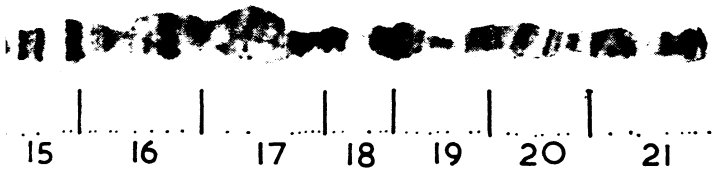
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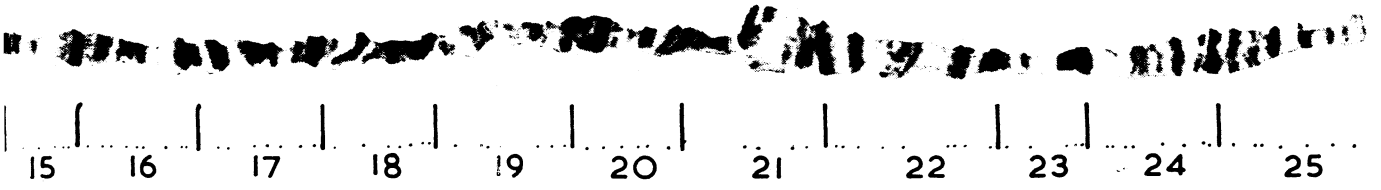
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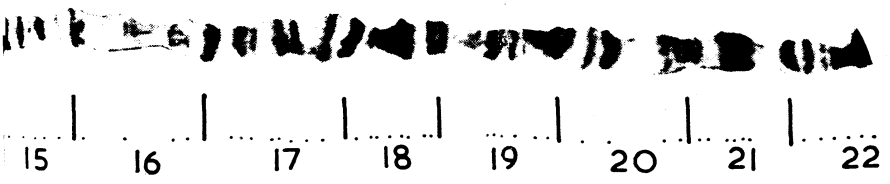
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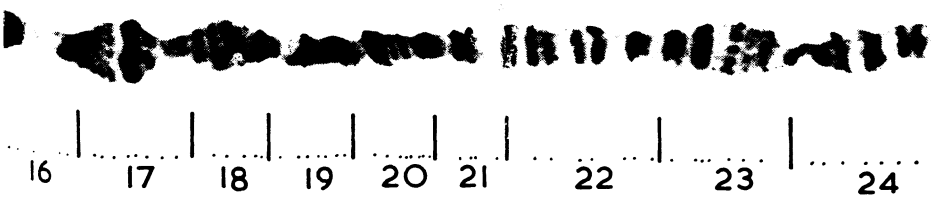
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They are labeled chromosome 1, representing the X chromosome; chromosomes 2L, 2R, 3L, 3R, and 4 represent the autosomes.

The Cairns strain was arbitrarily chosen as the standard sequence. A giant chromosome photographic map has been constructed by systematic photography of each chromosome arm under oil immersion and a series of overlapping prints were reconstructed in such a way that a more or less straight chromosome arm was obtained (Figure 2). Each chromosome arm is divided into sections, each of which contains not more than 10 major bands. The use of a giant chromosome photographic map and the system of numbering facilitate the accurate identification and description of breakage points and inverted segments.

Photographs of simple and complex inversions detected in the polytene chromosomes of *D. birchii* are shown in Figures 1, 3, and 4. At least 40 different inversions distributed nonrandomly in 5 giant chromosome arms have been found in samples from these localities, and they are described below.

Chromosome 1: The chromosome may be divided into two distinct regions. The distal region from sections 1 to 7 contains both simple and complex inversions. Two so-called "terminal" inversions have been found overlapping each other. The first terminal inversion R_1 (1.0–1.5) involves only five visible bands, in comparison with the standard sequence. Inversion R_0 overlaps inversion R_1 and its proximal breakpoint is at 1.7. The sequence of the section is: 1.7–1.6, 1.0–1.5. Following these inversions is a simple inversion A (2.3–5.0) involving 12 percent of the giant chromosome. This is followed by a further simple inversion B (3.2–5.4) involving 10 percent of the chromosome. Inversion C (5.5–8.0) apparently has the distal breakpoint the same as the proximal breakpoint on inversion B, and involves about 15 percent of the chromosome. Inversions D (4.6, 6.6) and E (2.7, 6.6) overlap inversion C. They have the same proximal but different distal breakpoints. The respective sequences of CD and CE overlapping complexes are: 7.0–8.0, 5.4–4.6, 6.6–5.5; and 7.0–8.0, 5.4–2.7, 6.6–5.5 (see Figure 2).

The proximal region ranging from sections 9 to 18 contains very complicated complex inversions, apparently all with the same proximal and distal breakpoints. These complexes involve about 50 percent of the chromosome. With respect to the standard gene arrangement the possible sequences of these complexes are:

P. 11.0–11.5, 10.0–10.6, 12.0–11.6, 9.9–9.0, 17.4–13.8, 18.1–17.5, 12.1–13.7.
S. 11.0–11.5, 10.0–10.6, 12.0–11.6, 9.9–9.0, 17.4–13.8, 13.0–12.1, 17.5–18.1, 13.1–13.7.
T. 11.0–11.5, 10.0–10.6, 12.0–11.6, 13.8–17.4, 9.0–9.9, 18.1–17.5, 12.1–13.7.
X. 11.0–11.5, 10.0–10.2, 13.7–13.2, 15.1–17.4, 9.9–9.0, 12.0–11.6, 10.6–10.3, 17.5–18.1, 13.8–15.0, 13.1–12.1.

Y complex is too difficult to analyze. Only a few cells yielding reasonably good giant chromosome-1 configurations were obtained among several hundred cells examined.

Chromosome 2R: Twenty-six different gene arrangements have been encountered, obtained as a result of random combinations of eighteen different inverted sections recognized in this chromosome (Figure 3). The distal part of the chromosome has a subterminal inversion A (1.4–4.1) involving 8 percent of the chromosome. This is followed by five simple inversions: B (2.8–6.2), C (4.2–5.5), D (7.1–9.4), E (11.6–24.2), and F (12.1–16.6), involving 12, 5, 11, 51, and 12 percent, respectively, of the giant chromosome. Four simple inversions have been found to be independently included within inversion E. They are inversions I (16.4–18.1), J (20.6–22.8), K (15.2–22.3), and L (19.2–22.5) and involve 7, 11, 30 and 15 percent, respectively, of the chromosome. Inversions G and M overlap inversion E with the breakpoints at (9.8, 15.3) and (11.1, 17.5), respectively. The respective sequences of EG and EM complexes are: 15.4–24.2, 11.5–9.8, 15.3–11.6; and 17.6–24.2, 11.5–11.1, 17.5–11.6. Inversion H (8.3, 13.0) overlaps both inversions D and E forming DEH complex with the sequence of 9.4–8.3, 13.1–24.2, 11.5–9.5, 7.1–8.2, 13.0–11.6. Inversion N is a second overlap on the DEH complex forming a more complicated inversion that has a sequence of 9.4–8.3, 13.2–21.2, 11.0–11.5, 24.2–21.3, 10.6–9.5, 7.1–8.2, 13.1–11.6. Two simple independent inversions O (12.3–15.2) and P (19.2–21.2) have been found in the Cairns population and each involves about 10 percent of the chromosome. Inversion Q (9.5, 20.3) overlaps inversion E forming EQ complex with the sequence of 20.4–24.2, 11.5–9.5, 20.3–11.6. Finally, simple inversion R (13.2–15.2) involves 7 percent of the chromosome and is included within EQ complex forming EQR sequence.

Chromosome 2L: Five inversions have been detected in this chromosome (Figure 4). A large single inversion A (4.2–16.4) involves 63 percent of the chromosome arm. Inversions B (16.6–19.5) and C (7.2–10.1) are respectively located at the proximal and distal regions and each involves about 15 percent of the chromosome. Inversion D overlaps inversion B and the breakpoints are at 10.2 and 17.4 and form BCD complex with the sequence of 10.1–7.2, 17.5–19.5, 15.4–10.2, 17.4–15.3. A simple inversion E (4.2–7.1) is located distal to inversion C, involving 12 percent of the chromosome.

Chromosome 3R: Two simple independent inversions A (5.1–10.0) and B (5.7–10.5) each of which involves 19 percent of the chromosome at the distal region and a large inversion C (10.6–19.6) involving 40 percent of the chromosome have been recorded (Figure 4).

Chromosome 3L: Two simple independent inversions A (5.1–10.2) and B (13.2–22.2) have been detected only at Daru and involve 21 and 46 percent respectively of the chromosome (Figure 4).

Chromosome 4 is free of inversions.

In addition, the presence or absence of an extra band or bands has been observed at the free end of chromosome 1. One and two extra terminal bands, in

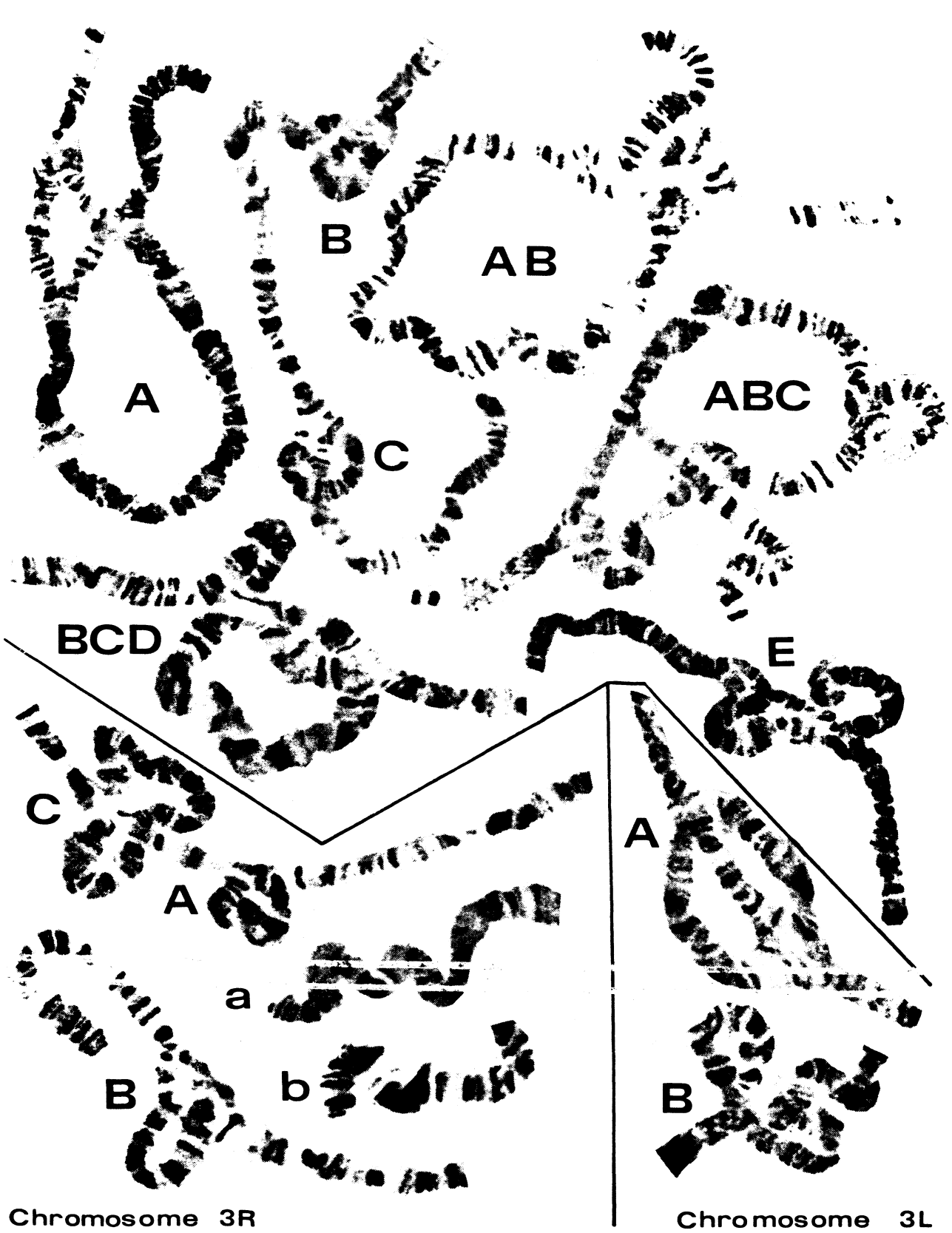

FIGURE 4—Heterozygous inversions in chromosomes 2L, 3R and 3L of *D. birchii*. The presence (a) and absence (b) of the terminal band of chromosome 3R are shown.

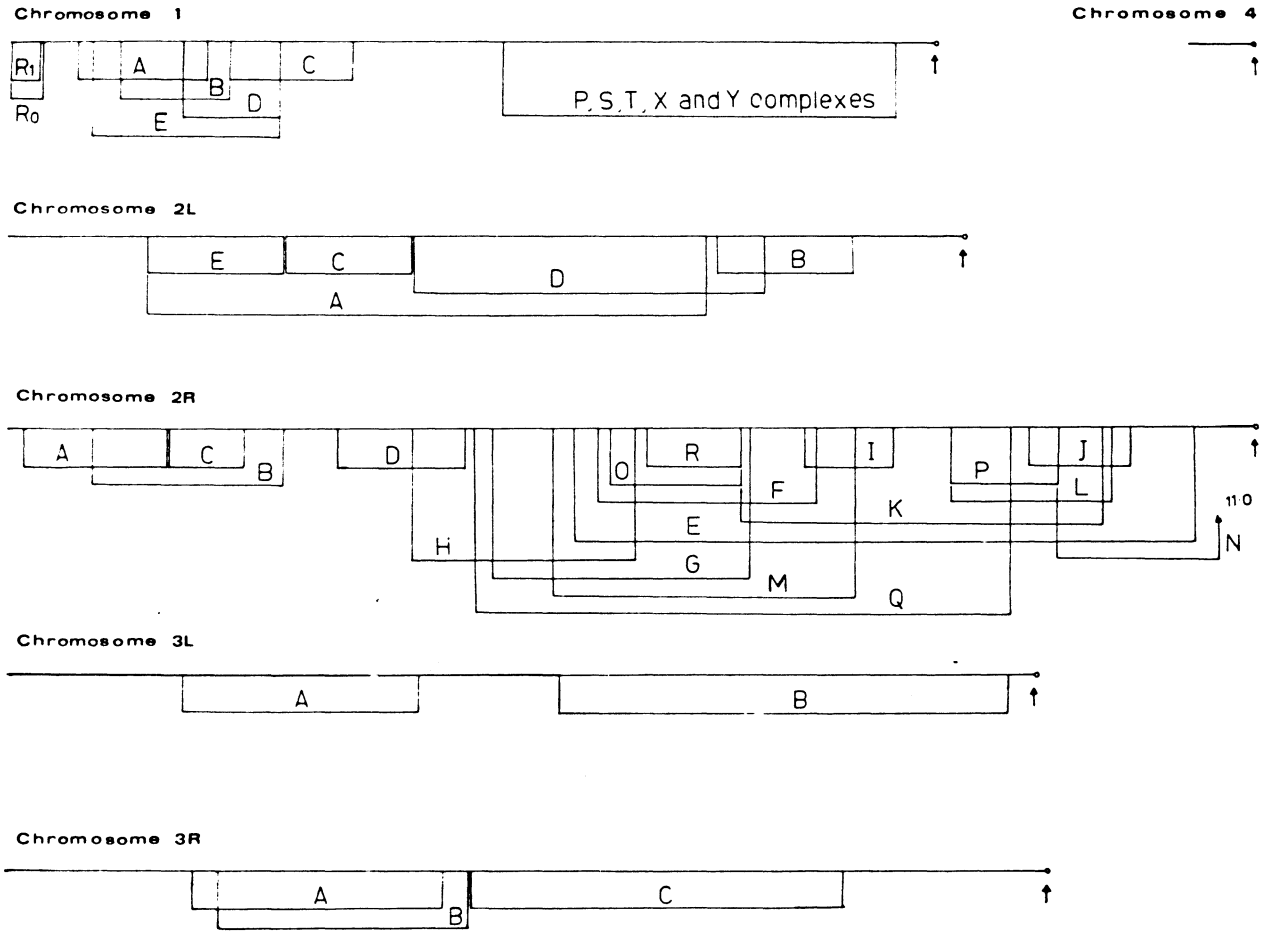

FIGURE 5—Diagram illustrating the relative lengths of the salivary chromosome arms of *D. birchii*. The limits of the known inversions are indicated by brackets. The centromere ends of the chromosome arms are indicated by arrows.

comparison with the standard chromosome, are present in addition to inversion R_1 (designated R_2 and R_3 respectively). Furthermore, an extra distinguishable band in section 10, with respect to the standard chromosome bands, has been observed. The presence or absence of the terminal band of chromosome 3R also has been encountered. It remains unknown whether such a peculiar phenomenon represents duplication or deficiency of that particular band.

The relative portions and locations of the known gene arrangements in *D. birchii* are shown in Figure 5. They are indicative of a pronounced concentration of chromosomal polymorphism in chromosome 2R and (to a lesser extent) chromosome 1, which manifests two clusters of inversions: very complex sequences in the proximal half of the chromosome, and simple and overlapping including terminal inversions in the distal region. Thus *D. birchii* exhibits a nonrandom distribution of inversions in the genome.

Inversion frequencies and distributions

The frequencies of gene arrangements found in natural populations are shown in Tables I, II, and III. For each gene sequence the percentage frequency as well as the number of occurrences (indicated in brackets) are given for each locality. The presence (+) or absence (−) of the corresponding gene arrangements at other localities where a few flies have been analyzed is also shown.

Chromosome 1: As may be seen from Table I, inversions C and R_1 are commonest in all localities of the species range except at Cairns where only the standard sequence has been found. Inversions A, B, D, and E have been found only in the territory of Papua-New Guinea. Of the complex inversions in the proximal region of the chromosome, P, S, T, and X complexes have been detected in the Bulolo population. However, T complex seems to be widespread in the territory while Y complex has been recorded only at Rabaul.

Chromosome 2R: As illustrated in Figure 3,

chromosome 2R is highly polymorphic. The frequencies and distributions of chromosome 2R gene arrangements are listed in Table II. As can be seen, inversion D is most common in all known localities including Cairns. Inversion E is also widespread throughout Papua-New Guinea and New Britain but is absent at Cairns. There is a strong indication of nonrandom association of these two gene sequences since inversion D has been found in the absence of inversion E only once at Port Moresby, and thirty-six times as BD sequence, and once as BDF sequence at Bulolo. It thus appears that the D sequence alone is nonadaptive in nature. The addition of new gene arrangements is therefore presumably in harmony with adaptation to natural conditions since they are common in many localities. Inversion A is also common in many localities and appears to be rigidly associated with the DE sequence. Nevertheless, it has been found only once at Bulolo (as ADEHN complex) while there is a marked increase in inversion frequency at Port Moresby and Popondetta. Inversion B, on the other hand, is significantly higher in frequency at Bulolo (2,500 feet) than at Port Moresby (1,500 feet at Bisianumu). Little is known about inversion C since it has been encountered only once at Port Moresby. Overlapping inversions G, H and M are unknown outside the area of Papua-New Guinea. Inversion H is significantly higher in frequency at Popondetta (at sea level), than at Port Moresby and Bulolo, whereas inversion G is more frequent at Port Moresby than Popondetta, but has not been de-

Table I. Frequencies (in percent) and distributions for chromosome 1 gene arrangements

Gene sequences	Moresby	Bulolo	Popondetta	Wewak	Daru	Rabaul	Cairns
Standard	—	—	—	—	—	—	+
R_0C	(13) 81.3	—	(65) 82.3	—	+	—	—
R_1C	—	—	—	—	—	(25) 100	—
F_1BC	—	—	—	—	+	—	—
R_2BC	—	(39) 86.7	—	+	—	—	—
R_3BC	—	(1) 2.2	—	—	—	—	—
R_0AC	(1) 6.3	—	(11) 13.9	—	+	—	—
R_1AC	(1) 6.3	(4) 8.9	(1) 1.3	—	—	—	—
R_2CD	—	(1) 2.2	—	—	—	—	—
R_0CE	(1) 6.3	—	(2) 2.5	—	—	—	—
P	—	+	—	—	—	—	—
S	—	+	—	—	—	—	—
T	+	+	+	+	+	—	—
X	—	+	—	—	—	—	—
Y	—	—	—	—	—	+	—
Chromosomes tested	16	45	79	3	5	25	3

Table II. Frequencies (in percent) and distributions for chromosome 2R gene arrangements

Gene sequences	Moresby	Bulolo	Popondetta	Wewak	Daru	Rabaul	Cairns
Standard	—	—	—	—	—	—	+
O	—	—	—	—	—	—	+
P	—	—	—	—	—	—	+
BD	(1) 3.1	(36) 40.1	—	—	—	—	—
DE	—	(3) 3.3	(4) 2.5	—	—	—	—
DP	—	—	—	—	—	—	+
ADE	—	—	(1) 0.6	—	—	(45) 90.0	—
BDE	—	(1) 1.1	—	—	—	—	—
BDF	—	(1) 1.1	—	—	—	—	—
DEG	(3) 9.4	—	—	—	—	—	—
DEH			(100) 97.1				
DEK	—	(1) 1.1	—	—	—	—	—
DEM	—	—	(2) 1.3	—	—	—	—
ADEG	(18) 56.3	—	(2) 1.3	—	—	—	—
ADEH	(8) 25.0	—	(40) 25.3	+	—	—	—
ADEI	—	—	—	—	—	(2) 4.0	—
ADEK	—	—	—	+	—	—	—
ADEM	—	—	(3) 1.9	—	—	—	—
ADEQ	—	—	—	—	+	—	—
BDEG	(1) 3.1	—	—	—	—	—	—
BDEH	—	(6) 6.7	—	—	—	—	—
BDEL	—	(26) 28.9	—	—	—	—	—
ADEHN	—	(1) 1.1	—	—	—	—	—
ADEIJ	—	—	—	—	—	(3) 6.0	—
ADEQR	—	—	—	—	+	—	—
ACDEH	(1) 3.1	—	—	—	—	—	—
BDEHN	—	(15) 16.7	—	—	—	—	—
Chromosomes tested	32	90	158	6	10	51	6

tected at Bulolo. Such variations in inversion frequencies may be interpreted in terms of altitudinal gradients.

Inversions F, L and N have been recorded only in the Bulolo population. Inversion K has been found at low frequency at Bulolo and also has been recorded at Wewak. Interestingly enough, inversions I and J have been recorded, at low frequencies, only at Rabaul. Likewise, inversions O and P are restricted to the Cairns population, while inversions Q and R so far have been found only at Daru.

Chromosome 2L: As indicated in Table III, this chromosome is polymorphic in all known localities in Papua-New Guinea, but is monomorphic at Rabaul (for BC sequence), and Cairns (for the standard sequence). Inversions B and C are widespread throughout the species range except at Cairns, while inversions A, D and E have never been found outside the Papua-New Guinea area.

Chromosome 3R: The standard sequence is significantly higher in frequency at Bulolo and Rabaul than at Port Moresby and Popondetta, while inversion C is more frequent at the latter than at the former localities. Inversion A, however, has so far been found in association with inversion C at Daru, whereas inversion B has been detected only at Cairns.

Finally chromosome 3L is known to be free of inversions throughout the species distribution except at Daru where, surprisingly, two independent inversions A and B have been encountered.

The mean number of heterozygous inversions per individual male is remarkably low in the Rabaul population (0.32 ± 0.69), while the populations in Papua-New Guinea are the most variable with mean inversion heterozygosity ranging from 1.62 ± 0.80 to 2.22 ± 0.89. These results thus appear to be in fair accordance with the hypothesis of da Cunha and Dobzhansky who have made extensive studies in the temperate species *D. willistoni*[9].

Phylogenetic sequence in chromosome 2R

The method of working out the phylogenetic relationships of the gene sequences in the chromosome is based on the system first developed by Sturtevant and Dobzhansky[23]. Use is made of overlapping inversions because their phylogeny may be inferred with a high degree of probability. Independent and included inversions, nevertheless, do yield additional phylogenetic information.

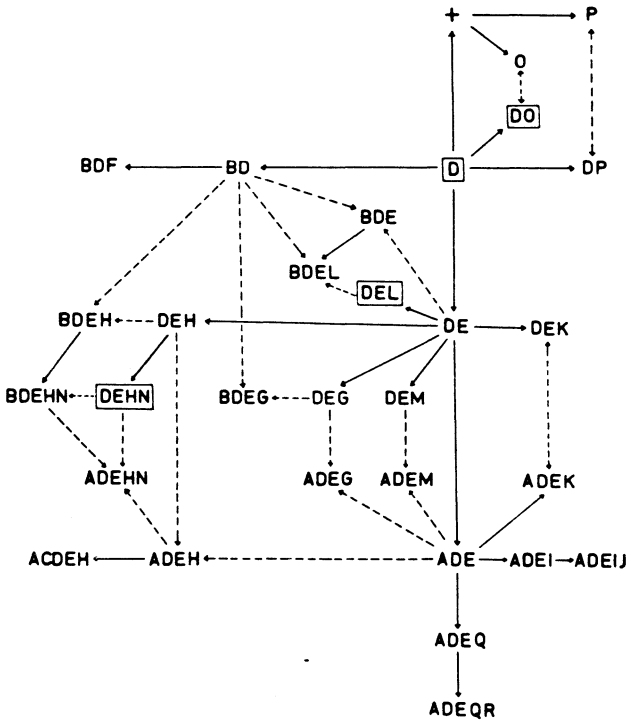

FIGURE 6—Phylogenetic scheme of chromosome 2R gene arrangements in *D. birchii*. □ = hypothetical sequence; + = the standard sequence. New gene arrangements by production of new inversions are indicated by solid arrows. Gene arrangements by recombination between the existing sequences are indicated by dashed arrows.

Table III. Frequencies (in percent) and distributions of gene arrangements in chromosomes 2L, 3R and 3L

Gene sequences	Moresby	Bulolo	Popondetta	Wewak	Daru	Rabaul	Cairns
Chromosome 2L							
Standard							
A	—	—	(1) 0.6	—	—	—	—
AB	—	—	(78) 49.4	—	—	—	—
BC	(10) 31.3	(16) 17.8	(3) 1.9	—	—	(50) 100	—
ABC	(22) 68.7	—	(63) 39.9	+	+	—	—
BCD	—	(74) 82.2	(11) 6 9	—	—	—	—
BCE	—	—	(2) 1.3	—	—	—	—
Chromosome 3R							
Standard	(3) 9.4	(89) 98.9	(8) 5.1	—	—	(50) 100	+
B	—	—	—	—	—	—	+
C	(29) 90.6	(1) 1.1	(150) 94.9	+	—	—	—
AC	—	—	—	—	+	—	—
Chromosome 3L							
Standard	100	100	100	100	—	100	100
A	—	—	—	—	+	—	—
B	—	—	—	—	+	—	—
Chromosomes tested	32	90	158	6	10	50	6

The available data have indicated that the D sequence is ancestral to the other gene arrangements including the standard sequence. Accordingly, the D sequence is taken as the starting point for the intraspecific phylogenetic scheme to be developed as follows (Figure 6).

Inversion D has never been found independently of association with other gene arrangements. Besides the O sequence detected at Cairns, the most simple gene arrangements found in wild populations are BD, DE, and DP. Hence it is highly probable that B, E and P could have arisen in the chromosome carrying the D sequence. The extensive range of the DE sequence in wild populations might suggest that it is well adapted to natural conditions. Inversion F is unknown outside the BDF sequence, which has been found only once at Bulolo. It thus seems likely that F could have originated in a BD chromosome.

Inversion A has always been found in association with the DE sequence. It seems probable that A could have arisen in the chromosome carrying the DE sequence.

Two possible pathways present themselves to explain the origin of inversion G which overlaps inversion E, i.e., D $\rightleftarrows$ DE $\rightleftarrows$ DEG but not D $\rightleftarrows$ DEG. However, the fact that D has never been found independently of association with other inversions and the DE sequence is commonest throughout the species range, suggests the D $\rightarrow$ DE $\rightarrow$ DEG pathway to be more probable than the other direction. Similarly, the origin of inversions H and M, each of which overlaps inversion E, can be specified as having arisen independently from a DE chromosome.

Inversions I and J are included in inversion E and have never been found outside ADEI and ADEIJ sequences that are apparently confined to the Rabaul population. Since the ADE sequence is most common in this population, it is highly probable that inversion I could have arisen in an ADE chromosome to produce the ADEI sequence in which inversion J subsequently originated. A somewhat similar situation is encountered in the Cairns population where inversions O and P including the standard sequence (+) are unique characteristics. Two possible pathways may be proposed for the origin of inversion P, i.e., D $\rightarrow$ + $\rightarrow$ P $\rightarrow$ DP (the last step by crossing over between the D and the P sequences) or + $\leftarrow$ D $\rightarrow$ DP $\rightarrow$P. At present it is not possible to say whether P arose from a D or a standard chromosome. Likewise, it remains uncertain as to the origin of inversion O. Although the DO sequence has not been found in wild populations, it might be expected to exist and be detected when a large number of samples are examined.

Inversion C has been found only once (as ACDEH complex) at Port Moresby. It seems unlikely that C had arisen in a DEH chromosome and subsequently recombined with an ADEH complex to give rise to ACDEH since its distal breakpoint is too close to (or perhaps overlaps) the proximal breakpoint of inversion A to allow the occurrence of crossing over. Further, the ADEH complex is common while the DEH sequence has never been detected in this population. Thus for this reason, inversion C could have arisen in an ADEH chromosome.

For BDE, BDEG, BDEH, ADEG, ADEH, and ADEM gene arrangements, each could have arisen independently as the result of recombination between the inversions already present in the natural populations, thus: BD + DE $\rightarrow$ BDE + D; BD + DEG $\rightarrow$ BDEG + D; BD + DEH $\rightarrow$ BDEH + D; DEG + ADE $\rightarrow$ ADEG + DE; DEH + ADE $\rightarrow$ ADEH + DE; DEM + ADE $\rightarrow$ ADEM + DE. The fact of the absence of the hypothetical independent inversion D, which occurred as the result of the first three recombinations, could indicate its nonadaptiveness in nature. The additional inverted sections and/or further recombinations could therefore have given greater adaptive values.

Inversion L is included in inversion E and is not known outside the BDEL sequence found at Bulolo. Two possible pathways may be suggested as the origin of L. It could have arisen directly from a BDE chromosome. Alternatively it could have originated in a DE chromosome to produce the DEL sequence that was presumably nonadaptive, since it has never been found in wild populations. In this case, recombination between a BD chromosome and the hypothetical DEL would have had to occur to produce a well adapted BDEL sequence if the newly formed L sequence were to survive. However, the former pathway seems more probable than the latter. A somewhat similar situation is found in inversion K, which is also included in inversion E. It has been found as DEK (at Bulolo) and as ADEK (at Wewak) only once each. It remains uncertain whether K arose in a DE or an ADE chromosome. In either case, recombination between the newly formed chromosome (DEK or ADEK) and the respectively existing sequence (ADE or DE) would have had to occur, thus: DEK + ADE $\rightleftarrows$ ADEK + DE. Nevertheless, the fact that inversions K and L are unknown in the south of the species range and are common in the north suggests that these gene sequences arose in the northern populations such as that at Bulolo.

Inversion N overlaps the DEH complex and has been found once as ADEHN, and sixteen times as BDEHN at Bulolo. Three possible pathways may be suggested as the origin of N. It could have arisen in a chromosome carrying either one of these complex sequences that include DEH, ADEH and BDEH. It seems unlikely that N arose in a DEH chromosome since DEHN has never been recovered in a wild population. If it did occur, a series of recombinations of the sort proposed in the origin of inversion L would have had to happen if the newly formed N sequence were to survive. On the other hand, the relatively high frequencies of the BDEH sequence in the population might suggest that N could have originated in this chromosome. Then the ADEHN could have come about as a result of recombination between the existing gene sequences. However, all three possible ways are shown in Figure 6.

Finally, inversion Q overlaps inversion E and so far has been detected in the ADEQ complex in the Daru population where a few flies were obtained and analyzed. At this stage, it may be assumed that Q could have arisen in an ADE chromosome. Inversion R is not known outside the ADEQR sequence; hence, it is likely that R could have arisen directly from an ADEQ chromosome.

Discussion

At least 40 gene arrangements have been found in 5 chromosome arms in *D. birchii*. This places it as one of the most polymorphic species in the genus. In fact, it is only exceeded by *D. willistoni*, which has some 50 inversions[9, 10], and *D. paulistorum*, which contains as many as 63 inversions[15, 16].

The polymorphism pattern in *D. birchii* shows some resemblance to that of the temperate species *D. pseudoobscura*, *D. paulistorum*, and *D. athabasca*, each of which has chromosomal variability limited to one chromosome. In *D. birchii*, the gene rearrangements appear to be concentrated in chromosome 2R and, to a lesser extent, in chromosome 1. The reasons underlying such nonrandom distributions of the inversions in the genome remain uncertain although several explanations are possible[21].

The gene arrangements in chromosome 2R are of particular interest since they permit inference to be drawn on the phylogeny of the gene sequences in the chromosome, and perhaps on the interspecific relationships among the members of the *D. serrata* complex. As indicated above, it is significant that inversion 2RD is most common and is presumably ancestral to the other gene arrangements in the chromosome. This might suggest that it could be a common gene sequence shared by closely related species. Hence, it would not be surprising to find an inversion in the corresponding chromosome in its sibling species that might be identical to inverion 2RD in *D. birchii*. A somewhat similar speculation may be implied to the 1C and 2LB gene sequences since they are widespread throughout the species range (except at Cairns).

Moreover, certain gene sequences in *D. birchii* are found to be confined to specific populations. Thus inversions 2RI and 2RJ are clearly limited to the Rabaul population, while inversions 2RO and 2RP have been found only at Cairns. Likewise, inversions 2RQ and 2RR including 3LA and 3LB are surprisingly characteristic of the Daru population. Besides the uniqueness of the gene arrangements mentioned, the metaphase plate morphology of chromosomes X, Y and 4 vary remarkably. However, certain gene sequences appear to run parallel with the chromosome types. Thus, for example, type-III Y chromosome so far has been found at Daru where inversions 2RQ and 2RR are present. In the case of the Rabaul and Cairns populations, these exhibit their own unique gene arrangements although they have the same metaphase chromosome types. The parallelism between the distribution of certain gene arrangements and various chromosome types found in *D. birchii* resembles the situation in *D. pseudoobscura*[11] and *D. athabasca*[20].

As a result of extensive studies of chromosomal variability in natural populations of the temperate species belonging to the *willistoni* group, Dobzhansky *et al.*[13] have advanced the working hypothesis that widespread species with populations inhabiting a variety of ecological niches should be more chromosomally polymorphic than those with relatively restricted ranges. This hypothesis has found support in the cytological evidence in *D. willistoni*[9, 10]. In contrast to *D. willistoni*, a widespread species throughout Central and South America, *D. birchii* is geographically restricted to the Australia-New Guinea area. Yet this species exhibits chromosomal polymorphism almost as great as that found in *D. willistoni*. The reasons underlying such a high degree of polymorphism in *D. birchii* are not clear. A tentative explanation, however, may be that the tropical rain forest environments could perhaps provide a variety of climatic and biotic conditions forming complex ecological niches. Consequently the development of complex systems of gene arrangements may have been in order to exploit such a variety of ecological niches and to expand successfully into varied environments. Alternatively, the chromosomal material in *D. birchii* might be innately more liable to breakage than is the case in other species particularly those from the same area. It is obvious that *D. birchii* is comparatively more chromosomally polymorphic than the well studied *D. rubida*[17] and *D. tetrachaeta*[1].

In the analysis of genotype frequencies of widespread gene arrangements of reasonably large samples of wild-caught flies from Port Moresby, Bulolo, Popondetta and Rabaul, no significant deviations from Hardy-Weinberg equilibria amongst the observed genotype frequencies were found. In fact the frequencies of heterozygous genotypes do not significantly exceed the 50 percent level.

Carson[7] and Mayr[19] have recently discussed the important role of isolated and marginal populations of a polytypic species in the process of race and species formation. The authors are of the opinion that geographically and ecologically marginal populations of a species tend towards monomorphism, while the central populations are characterized by more or less extensive polymorphism. However, a reduction in chromosomal polymorphism in the marginal population of the well studied *D. robusta* has been demonstrated by Carson[5, 6, 7], who has claimed that homoselection in the isolated peripheral populations facilitates the divergence of their genetic constituents, while heteroselection is more favorable in central populations in developing high levels of variability in adaptive gene complexes. Mayr has also pointed out that a marginal population is unlikely to give rise to a new species unless an effective barrier forms to prevent gene exchange between the marginal and the central populations. Such a barrier would permit the outpost population to evolve its own novel system of adaptive genotypes in natural environments.

In *D. birchii* a very marked decrease in chromosomal polymorphism has become apparent in the Rabaul population, suggesting marginal homozygosity for gene arrangements. This may be associated with ecologically and geographically marginal habitats, since Rabaul is widely separated from the territory of Papua-New Guinea by the water barrier of Vitiaz Strait. If this is the case, then it might be expected that similar associations would exist in the Cairns population, which is also geographically isolated from Papua-New Guinea by Torres Strait and is presumably near to the southern limits of the species range. The present data are indeed indicative that a low degree of polymorphism occurs at Cairns, since only a few different gene arrangements have been found in this locality. Furthermore, the unique gene arrangements existing in the Rabaul and Cairns populations may be equally well interpreted as the results of geographic isolation of the populations. Such physically and genetically divergent subdivisions of the species could ultimately lead to speciation. Further data will certainly throw light on this aspect of race or species formation.

Summary

A study of chromosomal polymorphism in *Drosophila birchii*, an Australasian member of the *melanogaster* species group, has revealed that this species is highly polymorphic. The giant salivary chromosomes consist of five long and one short arms. A standard stock was arbitrarily chosen from the Cairns strain. Samples from seven different geographic populations have uncovered at least 40 gene arrangements, with respect to the standard sequence. The inversions are preponderant in chromosomes 1 and 2R. Chromosomes 2L, 3L, and 3R exhibit only a few different gene sequences each. Little chromosome pairing in the proximal half of chromosome 1 takes place in hybrids between the standard strain and the others. Although many of the gene arrangements in *D. birchii* chromosome 2R are widespread, certain unique gene sequences have been encountered in the Rabaul, Cairns and Daru populations. Such unique inversions appear to run parallel with certain metaphase chromosome types. A phylogenetic tree in chromosome 2R based on overlapping sequences and inversion distributions makes it seem possible that the 2RD sequence is ancestral to the other gene arrangements and could be a common gene sequence shared by other closely related species. The variations of widespread gene arrangement frequencies between stations compared are apparently correlated with geographic differentiations and altitudinal gradients. The fact that the Papua-New Guinea populations, with the possible exception of the northern populations such as Wewak, show a high degree of chromosomal polymorphism while there is a marked decrease in the degree of structural variability at Rabaul (including perhaps at Cairns), suggests the ecologically marginal habitats in the latter populations resemble the situation found in *D. robusta* and *D. willistoni*. The results of the present study seem to furnish some support to the hypothesis that inversion heterozygosity dwindles towards the periphery of the species range.

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