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Chromosome Variability and Geographic Distribution in Insects

Chromosome rather than gene variations provide the key to differences among populations.

Bernard John and Kenneth R. Lewis

There is no group of organisms in which the analysis of chromosome variation in relation to geographical distribution has been carried further than in the Insecta. This kind of variation has tended to be neglected by many and ignored by some. Yet, in reality, it is more spectacular and, though less understood, at least as important as either the external variation or indeed the remaining genetic variation that exists in natural populations.

The chromosome complement is not just another character. To regard it as such is to misunderstand the nature of phylogenetic change and thus the whole basis of biological evolution. The material of the genotype itself forms part of the structure of the chromosomes. For this reason the chromosome phenotype is far less influenced by external factors than is the morphological or the physiological phenotype. Moreover, the chromosome phenotype is often a much more sensitive indicator of biological change and biological distinctiveness.

Chromosome variation of different kinds has been described within or between many natural insect populations (Table 1). In most cases, however, no analysis of this variation has been undertaken. The detailed study of chromosome variation in different geographical areas was initiated in the United States by the pioneer investigation of Dobzhansky and Sturtevant (*1*). They studied the polymorphism obtaining in the banding sequence of the giant polytene chromosomes of *Drosophila*. Their approach, that of comparing patterns of polymorphism within and between populations, has since been used by many workers in many lands and with many species. The *Drosophila* studies, however, still represent the most comprehensive and formidable body of data available on chromosome variation in geographically defined areas, for they have been in progress now for almost 30 years. Let us begin, then, by considering the extent, the validity, and the applicability of the conclusions reached from these studies.

Dr. John is reader in cytology in the department of genetics at the University of Birmingham, England. Dr. Lewis is university lecturer in botany and Fellow of Exeter College at the University of Oxford, England.

The Drosophila Affair

Populations of many, though by no means all, species of *Drosophila* are mixtures of individuals with differently constructed chromosomes. In particular, a large number of distinct paracentric inversions (Fig. 1) of the band sequences in the giant polytene chromosomes have been found in the heterozygous state, and each inversion tends to have its own definite range.

A few of these inversions occur throughout most of the species area, but most are restricted to varying degrees and some are quite local. The frequencies of some of the more abundant types vary along regular geographical clines and, since the clines do not coincide, each region is characterized by different frequencies of its principal chromosome types.

Different geographical populations of the same species may vary considerably with regard to their degree of polymorphism. Some are structurally monomorphic, some are moderately polymorphic, and others highly so. Moreover, in *D. subobscura* the inversions show a pronounced tendency to overlap, so forming complex heterozygotes, whereas in *D. willistoni* many of the inversions are small and independent.

Four main correlations have been established in relation to these varying patterns of polymorphism:

1) In species such as *D willistoni*, whose geographical races differ in the extent of their polymorphism, the chromosome variability of natural populations is highly correlated with environmental conditions. Populations in heterogeneous environments are more variable than those living in more homogeneous habitats (*2*). This suggests that chromosome polymorphism allows for a more efficient exploitation of the environment.

Table 1. Patterns of chromosome polymorphism within and between natural populations of insects. The preponderance of polymorphisms in the Coleoptera (beetles), the Diptera (flies), and the Orthoptera (cockroaches, grasshoppers, crickets, and mantids) undoubtedly reflects a lack of adequate study in other insect groups.

Type of polymorphism			Organism: Order	Organism: Species	Reference
STRUCTURAL	Paracentric inversion		Diptera	Many species of *Drosophila* but especially *melanica, melanogaster, pseudoobscura, persimilis, robusta,* and *willistoni*	(49)
				Chironomus dorsalis and tentans	(50)
				Glyptotendipes barbipes	(51)
				Anopheles punctipennis and *quadrimaculatus*	(52)
				Cnephia mutata	(53)
				Eusimulium aureum	(54)
				Simulium tuberosum	(55)
				Tenipes decorus	(56)
	Pericentric inversion		Coleoptera	*Pissoides approximatus, canadensis* and *terminalis*	(32)
			Diptera	*Drosophila algonquin*	(57)
				D. robusta	(58)
			Orthoptera	*Circotettix undulatus*	(59)
				Moraba scurra	(60)
				M. viatica	(61)
				M. virgo	(62)
				Scapsipedus aspersus	(63)
				Trimerotropis sparsa	(64)
	Interchange		Orthoptera	*Periplaneta americana* and *Blaberus discoidalis*	(27, 29)
	Centric fusion		Coleoptera	*Chilocorus stigma*	(35)
				Pissoides spp.	(32)
			Diptera	*Drosophila americana*	(65)
			Orthoptera	*Ameles heldreichi*	(36)
				Anaxipha pallidula	(66)
				Moraba viatica	(61)
				M. virgo	(62)
	Dissociation		Coleoptera	*Chilocorus spp.*	(35)
				Pissoides spp.	(32)
			Orthoptera	*Moraba scurra*	(60)
	Supernumerary chromosome segments		Orthoptera	*Calliptamus palaestinensis*	(67)
				Chorthippus parallelus	(39)
NUMERICAL	Aneuploidy	Supernumerary or B chromosomes	Coleoptera	*Diabrotica undecimpunctata*	(68)
			Hemiptera	*Cimex lectularius*	(69)
				Pseudococcus citri	(22)
			Orthoptera	*Acrida lata*	(70)
				Calliptamus palaestinensis	(71)
				Myrmeleotettix maculatus	(38)
				Trimerotropis sparsa	(72)
		Sex chromosome variation	Dermaptera	*Forficula auricularia* XY,XX/X_1X_2Y,$X_1X_1X_2X_2$	(73)
			Diptera	*Phryne cincta* XY,XX+(1—7) extra Y's	(74)
			Hemiptera	*Dicranotropis hamata* XY,XX/XO,XX	(75)
			Lepidoptera	*Solenobia triquetrella*	(76)
	Polyploidy		Coleoptera	*Scepticus griseus* diploid ♂ and ♀/ pentaploid parthenogenetic ♀	(77)
			Diptera	*Cnephia mutata* diploid ♂ and ♀/ triploid parthenogenetic ♀	(53)
	Undefined		Orthoptera	*Gryllotalpa gryllotalpa* 2 × ∝ 12, 14, 15, 17, 19 and 23	(78)

2) In a number of cases, inversion polymorphism is richest in the center of distribution and falls off toward the margins. This holds, for example, in *D. willistoni* when the average number of inversions per individual is used as an index of the structural diversity in a given population (*3, 4*). It holds also in *D. robusta* (*3, 4*), where the average length of euchromatin devoid of inversions increases from 65 percent in the center of the distribution to 85 percent in the marginal areas (Fig. 2). Similarly, by using an index of structural diversity similar to that of Carson, Stumm-Zollinger and Goldschmidt reported, contrary to earlier accounts (*5*), a higher index in the marginal populations of *D. subobscura* from Israel than in populations from central and western Europe. All three cases point to the same conclusion—geographically or ecologically marginal populations tend, on the average, to be less polymorphic than central populations.

The polymorphism of numerous populations of *D. willistoni* in the West Indies and in Central America follows the same rule. Not only does structural heterozygosity decrease with distance from the South American continent but, within the Archipelago, its extent is also clearly connected with the size of the islands (*6*). Thus island populations and those of distributional pockets are less polymorphic than continental populations. Likewise, when closely related species are analyzed, the ecologically more versatile prove to be more variable chromosomally (*7*).

Occasionally the interaction between such situations leads to what may appear to be paradoxical situations. Thus in *D. pseudoobscura* the chromosomal polymorphism is low in the populations of the Colorado plateau and the Great Basin, which are ecologically marginal though geographically central, and high in California and the Rocky Mountains, which are geographically marginal but ecologically hospitable (*8*). This implies that a species may evolve a specially adapted population in any ecologically "marginal" area, whether this is in the center of the species range or at its periphery.

Notice, however, that these conditions do not obtain in all cases. Brncic (*9*), for instance, finds that the chromosomally polymorphic species *D. pavani*, which lives in the ecologically diversified parts of Chile and Argentina, shows

no geographical differences in the frequencies of its chromosomal types. Likewise Kunze-Mühl, Müller, and Sperlich (*10*) found no reduction of the extent of polymorphism in island populations of *D. subobscura*, a situation which contrasts markedly with that discovered in *D. willistoni* on the islands of the Caribbean.

3) In some populations the chromosomal composition is known to undergo secular change. Thus cyclical, seasonal changes have been reported in the relative frequencies of the karyotypes of *D. pseudoobscura* on Mount San Jacinto and the Yosemite regions of California (*11*). On the other hand, in *D. willistoni*, which is the most widespread species in the genus and whose chromosome variability is the largest known, the inversions do not show seasonal fluctuations in frequency. It may be significant, however, that the inversions here are short and recombination between them is frequent (*12*).

4) Finally, the genetic composition of a population may change directionally with time. For example, between 1940 and 1957, populations of *D. pseudoobscura* from ten localities in different parts of California have all undergone a decrease in the frequency of the inverted gene arrangement CH in chromosome III with a corresponding appearance and increase in the frequency of the PP arrangement in the same chromosome (*13*). Sometimes the changes have affected populations over a very large territory, but attempts to correlate such changes with environmental variables have met with little success, and the causation of these changes remains an enigma.

Characters, Correlations, and Causations

Interesting as these findings are, the key question is—what confers adaptive significance on these different polymorphisms? There have been two main views on this subject. According to Dobzhansky, chromosomal polymorphism is maintained in natural populations of *Drosophila* chiefly by superior fitness of the structural heterozygotes for various combinations of the gene arrangements in a given population. This conclusion is based predominantly on the fact that in chromosomally polymorphic and monomorphic experimental populations, the polymorphic ones are fitter than the monomorphic if fitness is measured in terms of ability to convert nutrient medium into biological material. Polymorphic populations also appear to be superior in homeostatic properties (*14*). Rather surprisingly, however, no serious attempt has been made to elucidate the precise polygenic architecture of the inverted segments although techniques are available for doing this (*15*).

Epling and his colleagues (*16*), on the other hand, have repeatedly argued that the importance of these inversion systems depends on the restrictions and extensions to recombination which they effect. Of course, since crossing-over does not occur in the male of *Drosophila*, these restrictions and extensions will be immediately effective only in females. Thus genes within an inverted segment form a tightly linked constellation since even if recombination occurs between them the majority of the recombinants, being genetically unbalanced, will be inviable. Inversions in *Drosophila* also lead to interchromosomal influences on recombination, and different inversions differ in the intensity of their effect (*17*). Indeed, Epling

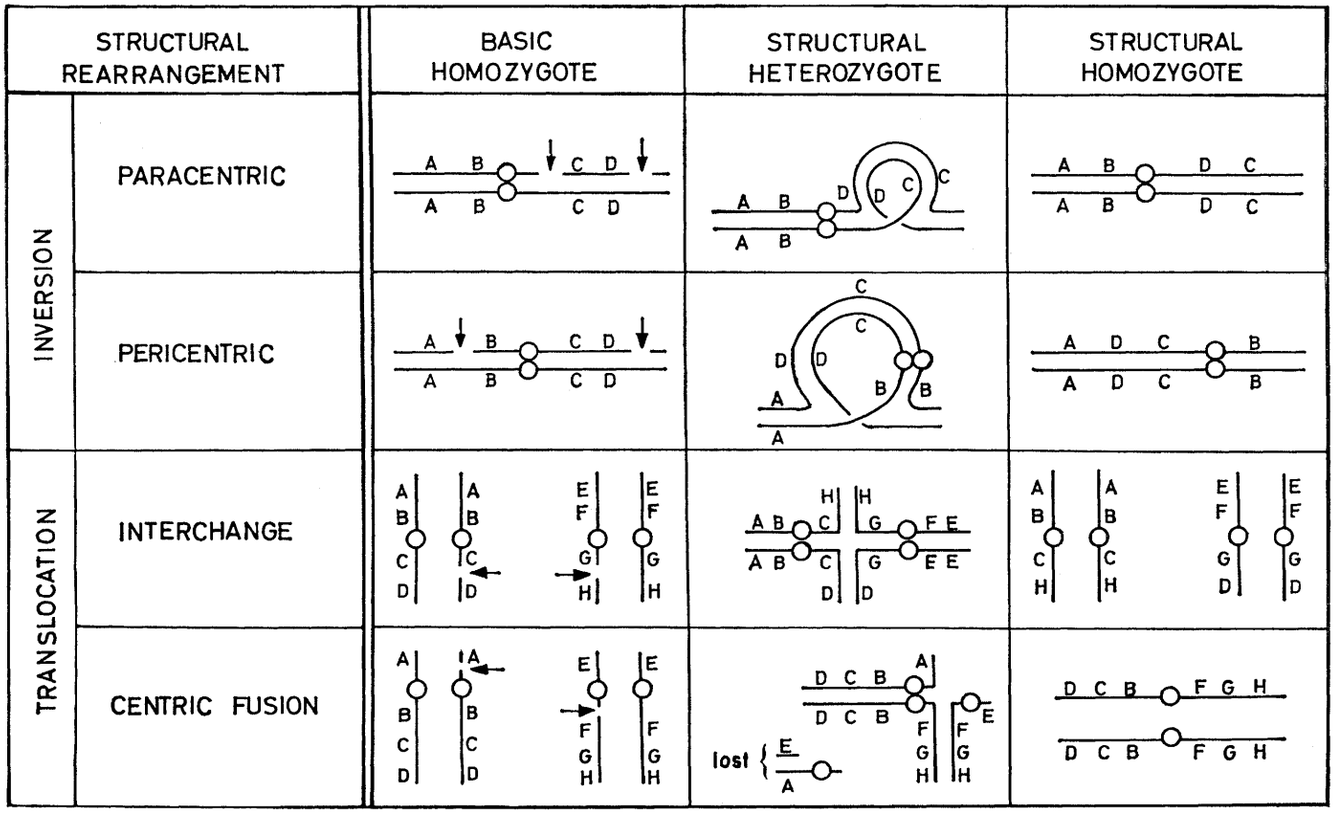

Fig. 1. The four principal types of structural chromosome mutation found in insect populations (compare with Table 1).

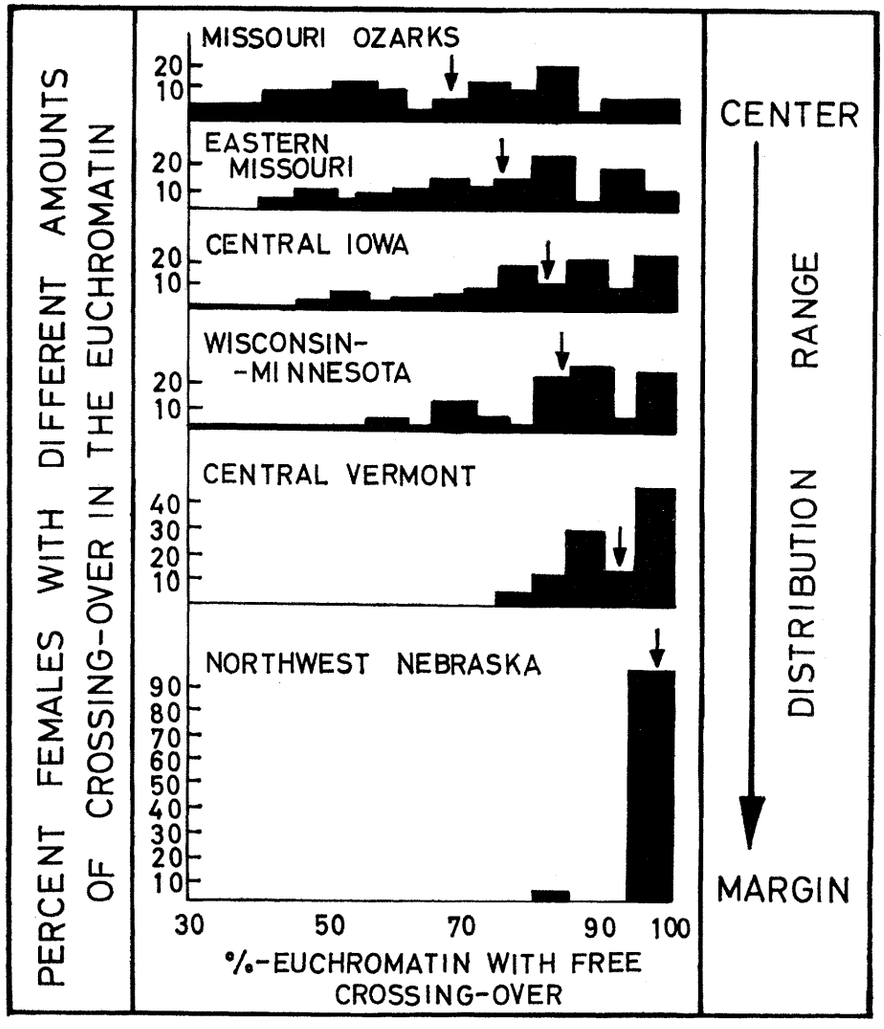

Fig. 2. Differential patterns of recombination in natural populations of *Drosophila robusta* as determined by Carson (*19*).

and his co-workers suggest that the seasonal changes of arrangement in chromosome III can be explained in terms of an increased recombination in genes other than those present in this chromosome. Moreover they believe that recombinants produced by crossing-over in the inversion-free chromosomes have effects on the adaptive values of the inversions themselves and that this influences their frequencies.

Likewise, two explanations have been advanced to account for the reduction of structural heterozygosity in peripheral populations. According to da Cunha and Dobzhansky (*3*) and da Cunha *et al.* (*18*) the gene arrangement has an ecotypic function. Carson (*19*), however, argues that the difference is due to selection for increased or decreased amounts of recombination. From his studies on *D. robusta* he has shown that the response to selection tends to be greater in strains originating from the marginal populations than in those from central populations. This undoubtedly reflects the occurrence of more recombination in the marginal populations.

Much of the difficulty in resolving the *Drosophila* affair undoubtedly stems from the fact that there is not one problem to solve but several. A second difficulty stems from the fact that it is not easy to distinguish effects due to genic heterozygosity from those due to recombination (*20*). A third arises from the fact that correlations need not be causations. Although it may be possible to demonstrate a clear correlation between a polymorphism and an environmental variable, such a correlation by no means proves that the variable is, or has been, instrumental in the establishment and maintenance of the polymorphism. Finally, there has been a tendency to confuse the properties of different levels of genetic organization. Thus conclusions relevant to simple gene heterozygotes—many of which in themselves are suspect—have been applied, without qualification, to chromosome heterozygotes. To take one example, inversions and interchanges, when heterozygous, produce tight linkage which leads to the development of what have been called "supergenes." And, provided this supergene combination shows heterotic properties, it may produce a system which simulates a simple gene heterosis. There are, however, very real differences between these two states. In essence we are dealing here with the question of the distinction between gene and chromosome mutation. It is true that this distinction is not absolute, but a comparison of these two kinds of mutation shows that it is not merely one of convenience. There are quite fundamental differences between them, differences we can conveniently summarize under four headings:

1) The magnitude of the change produced at the chromosome level bears no relation to the magnitude of its effect on the external phenotype, or exophenotype. Thus, in general, chromosome mutations, especially structural ones, cannot be detected in the exophenotype. There is, however, a second component to the phenotype, a component which includes, among other things, the behavior of the chromosomes themselves. And all chromosome mutations affect this aspect of the endophenotype, for they all interfere to some extent with the course, and hence the consequences, of meiosis. Where meiosis is abnormal, recombination and segregation are also likely to be abnormal.

Gene mutations as such, on the other hand, do not affect the course of meiosis, which is the same in genic homozygotes and genic heterozygotes. Chromosome mutations thus commonly modify meiosis in a way that gene mutations rarely do.

2) Most gene mutations are roughly recessive at their inception. Therefore, except in organisms whose principal vegetative phase is haploid and monokaryotic, they can persist, masked in the heterozygous state, even when their effects are harmful or indeed lethal. This means that they can be injected into new genotypes and dispersed *before* they are tested on phenotypic grounds.

Chromosome mutations, on the other hand, have their most pronounced endophenotypic effects in the condition in which they originate. A decision regarding the future of a chromosome mutation must therefore be made at the meiosis, or even at the mitosis, immediately following its origin. And the mutant must pass these mechanical tests before any of its other properties can be considered.

3) In general, as we have seen, chromosome mutations cannot be detected in the exophenotype. Even where they can, as in the case of position effects and polyploidy, the changes they determine are not usually different in kind from those that can be produced—and without the accompanying decrease in fertility—by gene mutations. This means that if selection takes the line of greatest fecundity it should, wherever possible, favor gene mutation as a basis for evolutionary change. Or, reversing the argument, we can conclude that where selection has favored chromosome mutation it has done so either because the innovation could not have been effected in any other way or else because it represents the only means of conserving an existing genetic regime in the face of changing circumstances.

4) Chromosome mutations may be maintained in populations by virtue of inherent mechanisms of accumulation at mitosis or at meiosis which establish systems of meiotic drive (*21*). Such systems need not be immediately useful, as Nur (*22*) has shown from his studies on the supernumerary chromosomes of the mealy bug. These chromosomes lower the "fitness" of the individuals possessing them under a variety of experimental regimes. Nevertheless, accumulation by mitotic nondisjunction appears to maintain them within natural populations. However, if these B chromosomes have an effect on variance, as Moss (*23*) has found in rye, they may confer longer-term advantages on the population.

These four principles have rarely been adequately recognized, let alone practiced. Thus many who have studied chromosome mutations have looked exclusively for exophenotypic effects and have ignored the endophenotype. Some few have even demonstrated an apparent exophenotypic influence and have then argued for a positive role for this influence in the evolution of the polymorphism in question.

The Moraba Affair

The small, wingless, Australian eumastacid grasshopper, *Moraba scurra*, exists as two geographically defined chromosome races with either 15 or 17 chromosomes in the male. This difference in number depends on the presence in the 15-chromosome race of a metacentric AB pair, both members of which are replaced in the 17-chromosome race by two acrocentrics A and B (Fig. 3). *Moraba scurra* is a species of the southern tableland of New South Wales and of Northern Victoria, and the two races differ in distribution. The one ($2n\ \delta = 15$) is eastern, whereas the other ($2n\ \delta = 17$) is western and much more restricted (*24*).

In both races the CD and EF chromosomes may exist in a number of forms, the commonest of which are shown in Fig. 3. And different populations may be polymorphic for different combinations of them.

White and Andrew (*25*) and White, Lewontin, and Andrew (*26*) find that at Wombat and Walendbeen (17-chromosome-race populations) and at Murrumbateman, Royalle, and Tarago swamp (15-chromosome-race populations) the Blundell (Bl) and Tidbinbilla (Td) chromosomes are size-decreasing while the corresponding standard (St and St′) elements increase the size of the individual (Table 2). White and his colleagues are of the opinion that this effect has a very definite role in the "genetic ecology" of the species, the origin and spread of the Bl and Td sequences having been coincident with the invasion of previously unexploited ecological niches. This conclusion is maintained although the two sequences show no apparent weight-reducing effect at Michelago.

Even accepting the correlation between chromosome constitution and size as a fairly general one, we may still legitimately question whether this correlation has anything to do with the maintenance of the sequences in the natural populations in question. Correlations are notoriously dangerous things with which to work. Consider, for instance, the situation in the plant *Oenothera*, where large sections of the genus are permanent interchange heterozygotes. These also contain a balanced system of recessive lethal mutations. But it is not the recessive lethals which determine the structural heterozygosity since, clearly, a balanced lethal system could not evolve in a structurally monomorphic situation. Rather, the development of a system of permanent structural hybridity has created a situation which allows for the accumulation of recessive lethals within the limits of the genetically differential segments that have been generated in the process (*27*).

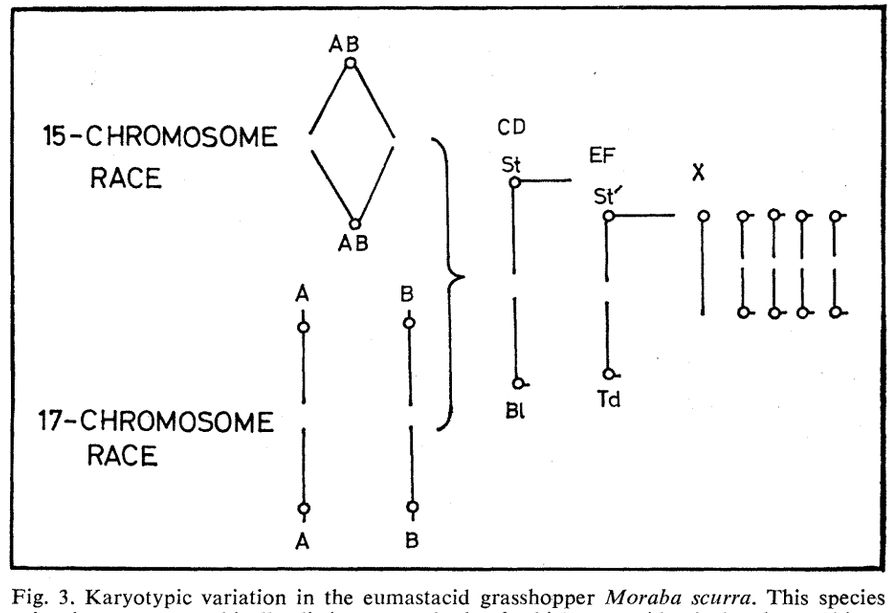

Fig. 3. Karyotypic variation in the eumastacid grasshopper *Moraba scurra*. This species exists in two geographically distinct races both of which carry identical polymorphisms with respect to the CD and EF chromosomes. White (*37*) believes these two races to be related by a process of dissociation rather than by centric fusion or centric fission (misdivision).

In *Oenothera* we have an organism which uses the processes and the structures designed for sexual reproduction in order to effect an essentially clonal reproduction. For meiosis and gamete development have been so modified that only two gametic types are produced, and these are identical with the gametes from which the parent itself arose. This is because, under the circumstances, truly sexual reproduction is prohibited by the unsuitable nature of its products, and we have here one of the classical examples of the conservation of heterozygosity (*28*).

The Periplaneta Affair

During our own work we have encountered a similar though less extreme situation in the American cockroach, *Periplaneta americana*. Here we have an approach to the system of permanent hybridity seen in *Oenothera*, for all populations of this orthopteran species contain a proportion of interchange heterozygotes (*29, 30*). That the situation is simpler here than in *Oenothera* can be explained by the fact that dioecious animals, unlike self-compatible monoecious plants, cannot inbreed so completely.

White and Andrew (*25*) have complained that no serious attempt has been made in this—or, indeed, other instances—to "go beyond the descriptive stage and analyze the adaptive role played by the rearrangement, although on general principles and by analogy with what is known as *Drosophila* we may suspect that heterosis (selective superiority of heterozygotes) or frequency-dependent selective values are involved."

That the structural heterozygotes are selectively superior in many cases of chromosomal polymorphism is evident. It is a question of where their superiority lies. By analogy with the situation in *Campanula* (*31*), we have argued that the interchange system of *Periplaneta* helps to conserve genic heterozygosity in the face of inbreeding. For interchanges decrease the frequency of production of the nonparental types which are expected to be subject to negative selection. In support of this thesis we have shown that the extent of structural heterozygosity is highest under conditions where inbreeding is also expected to be most marked (*29, 30*).

In this case, then, we are arguing that the principal role of the structural polymorphism is related to its effects on the distribution of genic hybridity between individuals in the population. Indeed, it could be argued that much of the chromosome variation which occurs in insect populations is related to the recombination process. This applies not only to structural mutations but to numerical ones too. And it applies also to cases which, though structural in character, are numerical in outcome.

The Pissoides and Chilocorus Affairs

A Robertsonian variation in chromosome number exists in the coleopteran genus *Pissoides* (*32, 33*). Here five species have a diploid complement of 30, three have 34, and one has 28 chromosomes (Table 3). In the 34-chromosome species all the members of the complement are acrocentric. Species with lower numbers have varying and proportionate numbers of metacentric elements. Thus two metacentric pairs (AA and BB) replace four acrocentrics [2(aa) and 2(bb)] in the 30-chromosome species, whereas the 28-chromosome forms have three metacentric pairs (AA, BB, and CC). In addition, three species, *P. approximatus, P. canadensis, and P. terminalis,* are chromosomally polymorphic, exhibiting a variety of karyotypes bridging the gap between the 28- and the 34-numbered species. And in different populations the frequencies of A and B metacentrics is subject to considerable variation.

In *P. terminalis* the polymorphism with regard to the C chromosome is complicated by its association with an incompatability system which is unique among animals (*34*). The males of this species are hybrid for the centric fusion [C(cc)] and for the sex-chromosomes (XY), so that four types of sperm are produced in equal numbers. The females, on the other hand, are homozygous for the fusion (CC) and for the X chromosomes (XX), so that all the eggs are of one kind. The system can perpetuate only if fertilization is confined to gametes which either (i) differ with regard to both the "auto-

Table 2. The mean net weight (W) in milligrams of (N) adult male individuals with various combinations of CD and EF chromosomes from five populations of *Moraba scurra*. Data from (*25*) and (*26*).

EF—CHROMOSOME	CD-CHROMOSOME: St/St	St/BL	BL/BL	St/St	St/BL	BL/BL	
	Wombat 1958–59			Wallendbeen 1959			
St'/St'	23	246	729	27	138	220	N
	34.28	33.18	32.75	37.06	35.37	34.51	W
St'/Td	3	65	271	6	39	57	N
	35.00	32.53	31.75	34.58	35.74	33.56	W
Td/Td	0	4	12	1	2	4	N
		32.63	29.25	38.00	39.25	35.25	W
	Murrumbateman 1959			Murrumbateman 1961			
St'/St'	107	359	262	39	194	132	N
	41.34	40.52	39.90	40.17	38.99	39.72	W
St'/Td	34	89	84	10	47	54	N
	39.91	39.03	37.56	40.60	39.07	37.91	W
Td/Td	0	3	5	0	1	3	N
		37.67	37.90		39.50	35.00	W
	Royalla 1958			Tarago swamp 1960			
St'/St'	22	95	75				N
	36.57	35.90	35.91	40.25	35.41	34.91	W
St'/Td	8	57	64	0			N
	35.69	35.65	35.34		35.00	35.08	W
Td/Td	0	6	6	0			N
		37.00	36.25		34.60	33.85	W

Table 3. Karyotype polymorphism in bark weevils of the genus *Pissoides*. Note a, b, and c represent acrocentric and A, B, and C equivalent—(aa), (bb), and (cc)—metacentric elements, respectively. Data from *(32)* and *(33)*.

Species	2x	Chromosome constitution (2x = 22+)
strobi engelmanni sitchensis	34	2(aa) 2(bb) 2(cc)
affinis fasciatus radiatae dubius notatus	30	AA BB 2(cc)
yosemite	28	AA BB CC
approximatus canadensis	30–34	2(aa) 2(bb) 2(cc) A(aa) B(bb) AA BB
terminalis	28–32	2(aa) A(aa) B(bb) C(cc), ♂ only AA BB CC, ♀ only

somal" fusion and the sex chromosomes or (ii) are similar in both these respects (Table 4). The *Drosophila* polymorphisms which we discussed earlier were investigated from salivary gland preparations, which means, of course, that males and females could be equally investigated. However, for technical reasons, most studies on insect polymorphism have been confined to the analysis of meiosis in males. But the *Pissoides* affair shows that one polymorphism can react with another, so that males and females may differ with regard to "autosomal" polymorphism.

Centric fusion is also found in the beetle *Chilocorus stigma* (*35*), where there is a sequential increase in the frequency of fusion chromosomes in populations running east to west on the North American continent (Table 5). Three distinct fusions are involved in this sequential polymorphism. One, the Kentville, is found in Nova Scotia and Maine. A second, morphologically distinct fusion occurs at Vineland, Ontario, while at Morden, Manitoba, a third fusion, again morphologically distinct, is found together with the more easterly pair. From their point of initial occurrence the frequencies of all three fusions increase steadily as populations progress north and west, so that this species is, in reality, a complex of cytologically differentiated forms.

In cases such as *Pissoides* and *Chilocorus*, chromosomal polymorphism exerts its control over recombination by virtue of the fact that it reduces the number of linkage groups and hence the extent of interchromosomal recombination at meiosis. Wahrman (*36*) has encountered a similar polymorphism for centric fusion in the mantid *Ameles heldreichi* in Jerusalem. Indeed here, as in *Drosophila*, there is a seasonal shift in the frequency of the structural types. That this is a genuine centric fusion and not a fission or dissociation process, as White (*37*) has suggested, is clear from Wahrman's observation (*36*) of a fusion *in statu nascendi*. The fusion had evidently arisen in the individual in which it was observed, for this male was mosaic and the small chromosome (Fig. 1) was still present at meiosis.

Centric fusion, then, is actually an unequal interchange which is followed by the loss of the small product. It has an effect on recombination comparable to that of the more usual type of interchange hybridity. The latter, however, can be successful only when approximately isobrachial chromosomes are involved, whereas acrocentric or telocentric chromosomes are more amenable to fusion. What is more, fusion is equally effective in both the homozygous and the heterozygous condition because, although the centromeres are not joined in the latter, they are linked by the first-anaphase movement to a common pole which balance demands.

The Truxaline Affairs

In the grasshopper *Myrmeleotettix maculatus*, British populations are polymorphic with respect to the presence of supernumerary or B chromosomes. These are completely absent from populations in Scotland and in northern England, but most of the southern populations contain a percentage of individuals with various combinations of from one to three supernumerary elements of two distinct morphological types. Where these extra chromosomes are present in a population they tend to raise the chiasma frequency of individuals which possess them above that found in normal representatives of that population (*38*). The same is true of supernumerary chromosome segments which are present in some British populations of a second member of the subfamily Truxalinae, *Chorthippus parallelus* (*39*).

Table 4. The incompatability system of *Pissoides terminalis*. Data from (*34*).

Egg	Sperm			
	X-type		Y-type	
	C	(cc)	C	(cc)
X, C	♀♀ XX, CC	Inviable combinations		♂♂ XY, C(cc)

In both these cases the presence of supernumerary, heterochromatic material leads to an increase in the range of recombinant types within a population. Indeed, variation in chiasma frequency itself probably represents one of the most common types of chromosome variability which exists in geographically distinct insect populations. And, since chiasma variation offers one of the most direct means of modifying the extent of recombination in an organism, one presumes that these variations are adaptational. Thus British populations of the grasshoppers *Chorthippus brunneus, Chorthippus parallelus, Myrmeleotettix maculatus,* and *Omocestus viridulus* all show significant differences in chiasma frequency, differences which are maintained from year to year, at least on a short-term basis (*40*).

The Locustana Affair

A few of the many species of the Acrididae possess a capacity for phase transformation which leads to the development of migratory swarms from solitary individuals. This transformation is reversible, so that a particular population of locusts may exist in the solitaria phase or the gregaria phase or in an intermediary transiens phase. The brown locust, *Locusta pardalina*, of South Africa is currently in a cycle of intensive gregarization after a period of some 7 years of predominantly solitary life. Gregarization began during the summer of 1962–1963 in a number of areas of the Karoo plateau, a region of some 260,000 square kilometers. Nolte (*41*) has compared the chiasma frequencies of different phases in populations from this region. He studied field samples of solitaria forms, congregating populations, and swarms with a history of one, two, or three generations of gregarization (Fig. 4 and Table 6). There has been no great mixing of

populations during the present swarming cycle, at least up to the end of 1963, and most of the collecting stations were at least 80 kilometers apart. Nolte found that in every case gregarization in the field led to an increased chiasma frequency and that this increase involved both long- and medium-type chromosomes. This increased frequency is carried over into laboratory offspring of field congregations and swarms. Moreover the phase status of X_1 individuals in cages does not seem to affect the increase.

There are, as Nolte in part points out, a number of internal discrepancies in the data which require more intensive study. However, if we accept his results at face value they indicate that in *Locustana* there is a progressive increase in chiasma frequency during the periods of gregarization which precede the outbreak of migrating swarms. Notice that this variation differs in three respects from that obtaining in most of the cases described earlier. First, it reflects genotypic and not structural control. Second, it is correlated with marked exophenotypic changes both in appearance and behavior. Third, although it is not directly related to existing spatial variation, it is followed by a process of migration.

Table 5. Sequential chromosome polymorphism in populations of the North American ladybird beetle, *Chilocorus stigma*. Data of Smith 1959 (31).

Region	Locality	Autosomal fusions per population (%)			
		1. Kentville	2. Vineland	3. Morden	Fusions
Florida	Moss Bluff and Minneola	Monomorphic populations—no fusions present $2x = 25\,♂\ (X_1X_2Y);\ 26\,♀\ (X_1X_1X_2X_2)$			
Atlantic seaboard	Kentville, Nova Scotia	8	0	0	Polymorphic populations $2X = 25\text{–}19\,♂$ $26\text{–}20\,♀$
Southern Ontario	Vineland, near Niagara Falls	14	6	0	
Central Ontario	Agawa River	27	36	0	
Manitoba	Morden	68	23	20	
Saskatchewan	Conquest	100 (fixation)	29	44	

Table 6. Mean chiasma frequencies among males in strains of the brown locust, *Locustana pardalina*, at various levels of gregarization in populations taken from the Karoo plateau. The population density is given as low when 3 to 40 individuals were counted in 400-meter transects. High-density populations have several hundred to 1000 individuals in equivalent transects. *P* indicates adults collected in the field and *X* indicates generations reared in cages under conditions of crowding from either egg pods or from offspring of field adults. Twenty diplotene cells were scored per male individual for each of 10+ males per strain. The male complement ($2x\ ♂ = 23 = 22+X$) consists of three long (*L*), five medium (*M*), and three short (*S*) autosomal pairs. The three *S* bivalents are not included in the scores since each invariably forms only a single chiasma. A difference in chiasma frequency of 0.3 to 0.4 indicates a probability of .001. Data from (*41*), and see Fig. 4.

Strain and symbol		Field collection		Generation scored	Chiasma frequency	
		Date	Population density		$3L_{II}$	$5M_{II}$
Matsup	Ma1	Nov. 1960	low	X_1 Mar. 1961	5.68	5.21
	Ma2	Feb. 1962	medium	*P* Feb. 1962	6.63	5.56
	Ma3	Dec. 1963	swarm	*P* Dec. 1963 X_1 Apr. 1964	7.44 7.36	6.97 6.60
De Aar	A1	Nov. 1960	low	X_1 Mar. 1961	5.40	5.07
	A7	Mar. 1963	swarm	X_1 Aug. 1963 X_2 Feb. 1964	6.99 7.37	6.37 6.61
	A8	Aug. 1963	swarm	X_1 Sep. 1963 X_2 Mar. 1964	7.11 7.33	6.43 6.68
Jansenville	Ja1	Oct. 1961	congregating	*P* Oct. 1961	6.47	5.82
	Ja3	Nov. 1961	high	*P* Oct. 1961	6.34	5.37
	Ja4	Jan. 1962	low	*P* Jan. 1962	5.91	5.14
	Ja6	Aug. 1963	low swarm	X_1 Oct. 1963 X_2 Mar. 1964	6.01 7.10	5.46 6.44

The Isolation Affair

It is relatively simple to show that the members of an outbreeding group are not genetically identical. With the exception of the chromosomes concerned with sex determination, the typical members of a mating group are far less often distinguishable chromosomally. Most of the heritable variation within a breeding group is thus unquestionably due to gene rather than to chromosome variation. When members of different species are compared, however, one often finds that the genetic differences between them are large enough to be detected by looking at the chromosomes. This is especially obvious when the chromosomes of their hybrids can be examined at the stages when maternally and paternally derived chromosomes pair. That is at meiosis in most organisms or in the polytene tissues of dipteran flies.

Now close adaptation to a given environment is possible only if the type favored in that environment breeds true to its own kind. In practice, the plant and animal breeder keeps his breeds apart, and it is this isolation that preserves their integrity. Nature is faced with the same problem of preventing gene flow between different groups, for evolutionary divergence is possible only when this flow is prohibited or at least curtailed. Chromosome heterozygotes frequently have one thing in common—an abnormal meiosis. Where meiosis is abnormal, semisterility can follow, and this semisterility is a potential barrier to gene

flow. Indeed, in all those cases which show stable chromosomal polymorphism, the structural heterozygotes must be able to pass through meiosis without producing an appreciable number of unbalanced gametes.

Chromosome changes of the kind that distinguish species must arise within species from chromosomally atypical individuals which are maintained in the heterozygous state by the pressure of mutation. And these floating variants, in turn, must arise from atypical cells in an otherwise normal individual. Grasshoppers illustrate this sequence nicely. Interchanges, for example, have been found in some cells within a testis, in some individuals in a population, and in hybrids between some geographically distinct groups (*42*). Indeed, any adequate survey of chromosome variation both within and between populations of insects leaves no doubt that there has been no shortage of either structural or numerical variation for selection to act on (*43, 44*). It is true that speciation involves discontinuity between individuals, but the development of discontinuity between populations depends, in the first instance, on that discontinuity arising within an individual.

It has long been argued, largely on mathematical grounds, that owing to the sterility of heterozygotes, there must be very strong selection against the establishment of structural changes in the homozygous state. Yet there are numerous cases which show quite clearly that such changes have occurred (*42*). Evidently here, as indeed elsewhere too, mathematical probability fails to accord with biological reality. Part of the problem in resolving this paradox is in deciding the precise role of the structural change relative to the production of the structural homozygote. As we have seen, structural changes are a common device for conserving the gene combinations of heterozygotes by protecting them from the ravages of recombination. They also offer one means of producing hybrid sterility. Many have assumed that it is the latter role which they perform in speciation. However, structural changes which behave regularly at their inception—and hence might be expected to reach fixation as structural homozygotes—are not likely to serve as effective isolating mechanisms. For their efficiency as isolating agents depends upon the meiotic inefficiency of their heterozygotes. It may be, therefore, that their importance in maintaining genic dissimilarity on crossing structurally differentiated chromosome homozygotes depends more upon the efficiency with which they maintain supergene blocks than on their capacity for producing hybrid sterility (*42*).

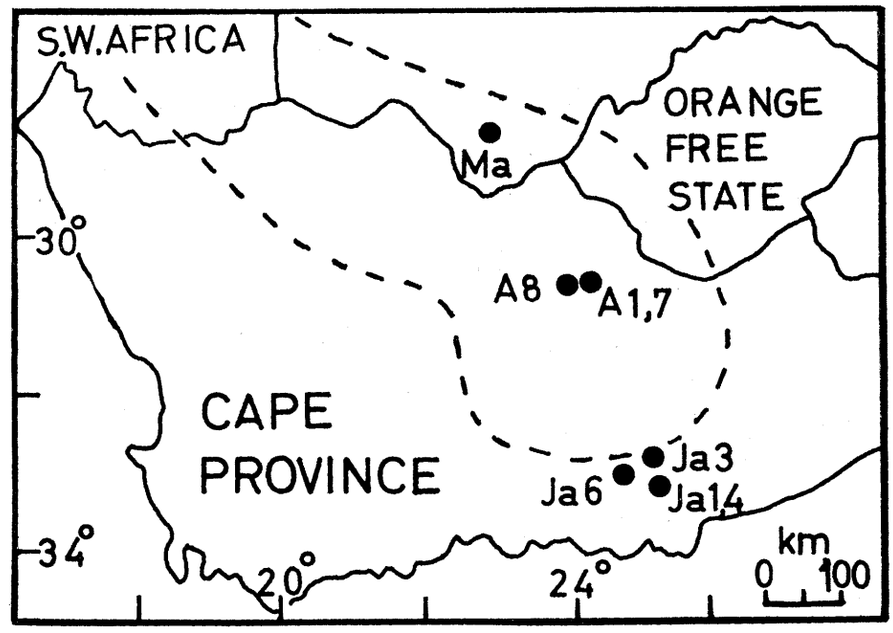

Fig. 4. The location of Nolte's (*41*) collecting stations for populations of *Locustana pardulina* taken from the outbreak region in the Karoo plateau (dotted line).

The difficulty of resolving whether a polymorphism can or cannot be supplanted by a monomorphism stems also from the problem of recognizing the correct role of structural change in natural populations. Despite earlier statements to the contrary, White, one of the foremost students in this field, has recently concluded that "not all the chromosomal rearrangements that establish themselves in evolution pass through a stage in which they are in a state of balanced polymorphism based on heterosis" (*43*). Instead, he believes that those which have not passed through such a polymorphic state must have arisen by chance drift in small local colonies probably situated on or close to the geographic periphery of the species range.

We have discussed the problem in detail elsewhere (*42*) and will say only this. The requirements for a successful polymorphic existence are usually quite distinct from those favoring the replacement of one monomorphic state by another. In time circumstances will, of course, change. But it does not follow that stable polymorphisms are necessarily sources of new, specific monomorphisms. Indeed, it is very clear that, in many cases, stable polymorphism is not, and cannot be, speciation in transit. And the kind of variation observed as a stable polymorphism within a species frequently differs strikingly from that seen between species related to it (contra *45*). Of course, species may arise from a previously polymorphic group, and they may differ from each other in the same way as the original morphic types. But "new differences would be superimposed on the polymorphism and would not spring essentially from it" (*46*).

The End of the Affair

Most of the new genes or chromosomes which arise by mutation are eliminated (stabilizing selection). They have, therefore, little effect on the species as a whole, and they pass undetected by the observer. Those that are favored may serve as focal points in the splitting of the mating group in which they arise (speciation), or they may persist within that group. In the latter event they can increase the variation potential of the breeding unit. At some time or place the new element may replace the old (directional selection), or disruptive selection within the breeding unit may produce two (or more) mutually dependent forms and so create a condition common to all stable polymorphisms. But, paradoxically, while retention of the new element can increase variation potential,

it may decrease the rate at which the existing potential is converted into free variation. This paradox goes a long way toward resolving the issue of whether a species is more variable at the margin or at the center of distribution.

An awareness of the distinction symbolized by the terms exo- and endophenotype adds a further dimension to the standard Darwinian argument, though even Darwin was aware of the difference. The endophenotype, by definition, does not affect the competitive efficiency or, therefore, the adaptedness of the individual; it affects the number and nature of the offspring and is, in consequence, the subject of retrospective selection. As an aspect of endophenotype what goes on at meiosis (or fertilization; compare *Pissoides*) has no meaning for the individual in which it occurs. This must hold for the various chromosome conditions which affect this division. And it is in this light that chromosome polymorphism must be viewed.

The essence of the argument is, again, seen most clearly in the ring-forming species of *Oenothera*, where the chromosome polymorphism within the breeding unit does not extend effectively into the zygotic phase. The ring-formers are clearly at an advantage. And, because their hybridity is at two levels, so also is their advantage. First, they are developmentally better: they alone survive (adaptedness). This must depend on the relational balance of the genotype. In this connection hybridity at the level of the karyotype does not matter as such: adaptation is a job for genes. But the ring-formers owe their genic hybridity to the fact that they are the structurally hybrid offspring of structurally hybrid parents and the parental genotype is transmitted more faithfully than it would be in the absence of a hybrid karyotype. Thus, genotypes make for good (or bad) individuals, karyotypes for good (or bad) parents. In the inbreeding, ring-forming species of *Oenothera*, genic heterozygosity and structural hybridity are absolutely correlated. Consequently the adapted individuals are also the best parents from the short-term point of view. *Drosophila tropicalis* approaches *Oenothera*, for there up to 90 percent of the adult population are hybrid (*47*).

Where, as in all the cases described earlier, structural homozygotes of various kinds survive and breed, the above correlation cannot be as close, because both homozygotes and heterozygotes, genic and chromosomal, can be derived from parental homozygotes or heterozygotes or both. But, clearly, selection will favor the restriction of recombination only if it favors the genotypes where restriction is practiced. Thus, in short-term evolution, under conditions of stabilizing selection the adapted (present fitness) genotype and the restricting karyotype will tend to go together. The nonrestricting karyotype, on the other hand, will go with the adaptational (future flexibility) genotype. And in these terms the "opposing" views on the *Drosophila* affair, for example, are reconcilable. We see, therefore, that while the genotype matters to the individual, the karyotype does not—so long as it is mitotically competent. But the karyotype matters to the unborn and thus to the population.

In those cases where it has proved possible to analyze the situation in some detail, there are good grounds for arguing that the chromosome variability shown within and between insect populations is concerned with the regulation of recombination. Clearly, recombination has a meaning only in hybrids. It is not easy, therefore, to distinguish the effects of heterozygosity from those of recombination. And, whereas recombination can be concerned only with adaptability, heterozygosity must be concerned in both adaptation and adaptability. We can be warned, however, that it is not the sequence of genes which linkage protects that matters but the relations beween these genes and those on the partner chromosome. Where, as for example with inversions, alternative sequences are internally balanced but in different ways, a restriction of recombination is important. But, under these same circumstances, structural homozygotes are similar with regard to nonallelic interaction of a polygenic kind. Perhaps this situation is approached in those *Drosophila* polymorphisms which do not show spatial or temporal variation.

Where, on the other hand, alternative sequences are balanced only in relation to each other, structural homozygotes will show less balance. They may, however, increase under directional or disruptive selection. Perhaps this situation is approached in those cases where the chromosome polymorphism does change either cyclically or directionally with time and place.

Conclusion

Mayr has recently claimed (*45*) that "no substantial work dealing with the better known groups of animals fails to include information on the geographical variation of the species treated." Unfortunately, most such works regularly fail to include information on chromosome variation. It is our hope that this review will serve to show that, as far as the study of geographical distribution in insects is concerned, it is clearly time to examine more fully not the exophenotype but the endophenotype, not the obvious and external but the microscopic and internal, not the genic but the chromosomal. For as Darlington (*48*) has so aptly put it, "While marker genes, the chief legacy of classical genetics, with their pedigrees and their mutation rates, are of great importance for the study of evolution, they are of little importance in carrying it out."

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Plant Hormones and Regulators

Gibberellins, cytokinins, and auxins may regulate plant growth via nucleic acid and enzyme synthesis.

J. van Overbeek

Just about 40 years ago, in opposite parts of the world, proof was given of the existence of substances which promote growth of plants. In 1926 Went (*1*), in Holland, provided convincing proof of a diffusible substance obtained from oat seedlings which promoted growth of these seedlings. This was the beginning of auxin research.

Kurosawa in Japan, in the same year (*2*), gave proof of a substance in cell-free fungus filtrate which promoted growth of rice seedlings. This was the beginning of gibberellin research, although the Western world did not take notice until the early 1950's. Auxins and gibberellins are now recognized to be two separate classes of chemicals that cause distinct growth patterns in plants.

Auxins

It is now reasonably certain that the native auxin is indole-3-acetic acid (IAA, Fig. 1) (*3*). Indole-3-acetic acid occurs in minute quantities in growing tissue. Thus, in the shoot of the pineapple plant, only 6 micrograms of auxin are found per kilogram of plant material (*4*). J. P. Nitsch (*5*) calculated that this is like the weight of a needle in a 22-ton truckload of hay. One reason that this concentration is so low is that IAA is constantly being destroyed by indole-3-acetic acid oxidase (*6*). This enzyme system definitely occurs in intact plants (*7*). Indole-3-acetic acid oxidation is usually activated by monophenols and inhibited by orthodiphenols (*8*). Recognition of this fact has clarified the growth-promoting activity of diphenols such as caffeic acid. Previously, they were thought to be auxins; now it is recognized that, by inhibiting the IAA oxidase, these compounds raise the level of native IAA considerably (*9*). This is a form of synergism.

Many synthetic auxins (*10*) have been found. Some of these have a biological activity more potent than that of IAA, probably because they are more persistent in the plant than this native auxin is. The best known of these synthetics is 2,4-dichlorophenoxyacetic acid, the herbicide 2,4-D (*11*). In the United States alone, this chemical is now produced at a rate of over 100 million pounds (45 million kilograms) per year (*12*).

Although synthetic auxins are more stable in plants than the native auxin is, synergism is still found among them. Thus, Veldstra (*13*) reports that the activity of 10^{-6} mole of naphthaleneacetic acid could be increased 40-fold by supplementation with 2×10^{-5} mole of decahydronaphthaleneacetic acid, which is inactive by itself.

Auxins are required for cell elongation as well as for cell proliferation, but they have a multitude of additional effects. In tissue cultures, the native IAA is often replaced by 2,4-D, as 2,4-D is more stable and less likely to undergo biological degradation (*14*).

The author is chief plant physiologist, Agricultural Research Division, Shell Development Company, Modesto, California.