Species of Drosophila

New excitement in an old field.

Theodosius Dobzhansky

The species problem is the oldest in biology. Adam was allegedly called upon to distinguish and name species in the Garden of Eden. Preliterate people faced this task wherever they lived. Some of them were perspicacious naturalists who recognized mostly the same species as zoologists and botanists do, while others were less successful (*1, 2*). To Linnaeus and his followers, species were the basic constituents of the living world, the primordial created entities. Variations within a species were merely imperfections in the manifestation of the immutable archetype (Platonic eidos), of which every species was the embodiment. Evolutionists showed that species are not separated by unscalable walls. Intraspecific variations are important as raw materials from which race and species differentials are compounded in the process of evolution. Lamarck and Darwin stressed that species were not all uniformly discrete. Darwin concluded that "species are only strongly marked and permanent varieties, and that each species first existed as a variety" (*3*).

To classical taxonomists, species and races (varieties) were categories of classification. To students of evolution they are also biological phenomena. Much confusion would be avoided if this duality of the species concept were understood clearly. A biological species concept is therefore necessary. Its beginning goes back to John Ray, who stated in 1686 that "one species never springs from the seed of another" [quoted in (*1*)]. Attempts were made later to define species as forms that are unable to cross, or that produce inviable or sterile offspring when crossed. These definitions are valid as far as they go, but they do not go far enough. In the light of the biological (synthetic) theory of evolution it became clear that, in sexual and outbreeding organisms, species are reproductive communities. They are separated by any one, or by a combination of several, reproductive isolating mechanisms, (*1, 4–7*). Hybrid inviability and sterility are among such mechanisms, but there are others (for example, ethological and ecological isolations) which may be just as effective in nature. The recognition that species are reproductive communities was a step forward, but it raised more problems than it solved.

Multiformity of Species

Sexuality is a widespread method but not the only method of reproduction. There are organisms that are exclusively asexual, parthenogenetic, or self-fertilizing. The species concept based on reproductive isolation is inapplicable in such organisms. Arrays of structurally and ecologically diverse forms may nevertheless be present; systematists describe some of the arrays as races, others as species, genera, and so on. This is legitimate for cataloging purposes, but which arrays are to be designated species becomes arbitrary. "Species" in asexual or parthenogenetic forms are really pseudospecies, biological phenomena unlike species in sexual outbreeders. The contrast may, however, turn out to be less radical than it seems, owing to the discoveries of parasexuality, transformation, and transduction in organisms which were believed to be strictly asexual (*8*).

The genetic nature of species is a function of the reproductive biology of the organism concerned. Allopolyploidy, chromosome doubling in species hybrids, may lead to sudden emergence of reproductively fully discrete and sympatric neospecies. More often a species is transformed into a new one in time, or gives rise to two or more derived contemporaneous species, by gradual accumulation of genetic differences. The divergence of the incipient species occurs, as a rule, allopatrically, in geographically separate populations (*1*). Because the process of speciation is gradual, one expects to find instances of populations between which the gene flow is incompletely repressed. Such instances have indeed been found, and they have led to a misapprehension that all species are arbitrary groupings. This is not so. Borderline situations between race and species are, on the whole, rare; they may be annoyances to classifiers but are precious to experimental evolutionists.

Somewhere between 1.5 and 2 million species have been described and named. Species are not only numerous but of many kinds. Neither the classical typological nor the more modern biological (populational) species concept accommodates easily the great, and until recently scarcely appreciated, diversity of the evolutionary patterns in the animal, plant, and prokaryote kingdoms. Evolutionary biologists are challenged to reexamine the old and to look for new approaches. A new consensus is yet to emerge. Excitement and clash of opinion have returned to to the field, which at times during its long history threatened to freeze in an orthodoxy. Some feel that species must have the same meaning in sexual and asexual, prokaryote and eukaryote, contemporaneous and allochronic organisms. This procrustean operation sacrifices reality for generality. Others underestimate the role of reproductive isolation in evolution, and claim that: "For sexual organisms it is the local interbreeding population and not the species that is clearly the evolutionary unit of importance" [(*9*); see also (*10*)]. Still others, and I believe their approach is heuristically the most productive, stress that species are not all of the same kind, and different sorts of species should be understood in relation to different strategies of evolutionary adaptation.

Only a fraction of the evolutionary patterns that exist in the living world are encountered among drosophilid flies. Yet they are diverse enough and, owing to many drosophilids being favorable for experimental work, they can be studied in depth. The highlights of these studies may, I believe, be of general interest.

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"Good" Species

There are probably no fewer than 2000 species of drosophilid flies (*11, 12*). Species that live in the same territory (sympatric species) are generally more numerous in the tropics than in temperate and cold zones. More than 100 species of *Drosophila* have been recorded for the republic of Salvador, 39 in one locality in Texas, only 9 in Alaska, and a single species introduced by man in Tierra del Fuego. The archipelago of Hawaii, with total area smaller than the state of Massachusetts, has over 500 species of drosophilids, all but 17 of which are endemics (*12*).

It can easily be shown that species of *Drosophila* are biologically real, not arbitrary, entities. With rare exceptions, sympatric species are reproductively fully isolated in their natural habitats. The isolation usually persists in laboratory environments as well. Taking almost any pair of sympatric species, one finds as a rule not one but several isolating mechanisms in operation, and often different ones between different species. Because species have distinct food and microhabitat preferences, conspecific individuals meet more often than individuals of different species. More widespread and more potent than this ecological isolation is ethological (behavioral, sexual) isolation. Comparative studies of courtship and mating habits in drosophilid flies have been pioneered by Spieth (*12, 13*). Males of many species are promiscuous, in the sense that they approach any moving object of about their size. "Tapping" with foretarsi leads, however, to recognition of the species and sex tapped. Females have rejection signals whereby they may repulse males of their own and foreign species. Species-specific courtship and mating patterns are amply diversified. The diversity is especially prominent in Hawaii, where sympatric species are most numerous. This is as it should be if premating isolating mechanisms are mostly not accidental by-products of genetic divergence but adaptive contrivances that guard against breakdown of functionally coherent adaptive systems.

Ethological isolation may be incomplete under laboratory conditions between the same pair of species that are completely isolated in their natural habitats. Hybrids of *Drosophila pseudoobscura* and *D. persimilis* are easily obtainable in the laboratory, but they are absent in localities where both species occur side by side (*14*). Mechanical isolation, noncorrespondence of the genitalia, is found also more often in the laboratory than in nature. *Drosophila melanogaster* males that attempt to copulate with *D. pseudoobscura* females may become locked together, and die unable to separate. I found such a couple in nature. Hybrid inviability and sterility are, for obvious reasons, hard to ascertain in nature. Hybrids of *D. melanogaster* and *D. simulans* are inviable either as females or as males (depending on the direction of the cross), and the surviving sex is sterile. Yet these species occasionally cross in the laboratory as well as outdoors (*15*). *Drosophila pseudoobscura* and *D. persimilis* are often sympatric; only four females inseminated by foreign species, and one hybrid, have been taken in nature among thousands studied. Conclusive evidence that these species do not effectively hybridize in nature comes from studies on their chromosomal polymorphisms. Both species are highly polymorphic for chromosomal inversions, but only a single, presumably ancestral, gene arrangement is common to both species. Other inversions are species specific. No individual of *D. pseudoobscura* has ever been found carrying an inversion characteristic of *D. persimilis*, or vice versa. Either these species do not cross in nature at all, or if they do the foreign genetic material is promptly cast out by natural selection (*16*). Similar, although less extensive, evidence of lack of gene exchange is available for sibling species of the *D. willistoni* group [compare (*17*) and (*18*)].

Sibling Species

Pairs or groups of species that are morphologically indistinguishable, or distinguishable with difficulty, are called sibling species (*6*). Although known in many groups of organisms, sibling species are notably common among drosophilids. It can reasonably be inferred that sibling species are genetically more similar on the average than morphologically distinguishable species in the same group of organisms. Yet it should not be assumed that sibling species always represent examples of uncompleted speciation. As pointed out above, *D. pseudoobscura* and *D. persimilis* are in nature completely isolated reproductively. Their males can be distinguished by a minute difference in the genitalia (*19*), and females solely by means of genetic, cytological, and biochemical tests (*16, 20*).

Some museum taxonomists as well as modern computer classifiers refuse to recognize sibling species because they cannot distinguish them. Yet sibling species are important to evolutionists; they permit dissection of the process of speciation into studiable components. Sibling species and ordinary morphologically distinct species are biologically similar phenomena. Visible morphological distinctions between "good" species range from very striking to none. Reproductive isolation evidently can arise with little or no morphological differentiation. Whether or not it can arise also with little physiological and adaptive differentiation is another problem that I shall discuss below. It is remarkable that despite explosive proliferation of drosophilid species in Hawaii, most of them are morphologically distinct, and sibling species are rare (*12, 21*). In contrast, the *willistoni* group consists of at least six sibling species, four of which are widespread, common, and sympatric over much of tropical America (*22*).

Superspecies and Semispecies

While sympatric species, including siblings, are as a rule unambiguously distinct, the same cannot be said of allopatric, geographically separate, populations. A variety of situations are encountered. *Drosophila willistoni* and *D. paulistorum* are contrasting examples. They are reproductively isolated sibling species. But while the former is a simple, nearly monolithic species, the latter is a complex superspecies on the verge of breaking up into at least five derived species. The distribution of *D. willistoni* extends from Mexico and southern Florida to Argentina (La Plata). With a single exception, strains from anywhere in this tremendous territory interbreed freely and give fertile hybrids (*23*). The exception is a strain from Lima, Peru. The Lima strain crosses easily with all other strains with which it has been tested. Crosses in which Lima is the male parent give fertile hybrids of both sexes. The reciprocal crosses also give fertile hybrid females. Male hybrids are also fertile when Lima females are outcrossed to strains from Mexico, Central America, and Ecuador, but sterile in outcrosses to Brazilian, Trinidadian, and Colombian strains (*24*). It would be gratuitous to regard Lima anything other than a member of the species *D. willistoni.*

Drosophila paulistorum is distributed less widely than *D. willistoni* (from Guatemala to southern Brazil). It is a superspecies composed of five semispecies. The semispecies are nearly identical morphologically (*25*), and yet they have evolved reproductive isolation sufficient to enable them to coexist sympatrically in some places, apparently with little or no hybridization (*22, 26*). Laboratory tests show a strong, though incomplete, ethological isolation; females usually rebuff males of all but their own semispecies. Each semispecies has a courtship ritual somewhat different from the others (*27*). When cross-insemination nevertheless takes place, vigorous hybrids are produced which are fertile as females but completely sterile as males. Every semispecies has a geographic distribution different from the others, and yet while in many places only a single semispecies is found, from the Amazon Valley to Panama two or even three semispecies live together.

Sympatric coexistence of populations without gene exchange is prima facie evidence of a speciation process having been completed. A cogent argument can be made that *D. paulistorum* is really a set of five species. On the other hand, the incompleteness of the ethological isolation and the unimpaired fertility of female hybrids suggest that some gene exchange between the semispecies may be taking place. As pointed out above, examination of the chromosomes in populations of *D. pseudoobscura* and *D. persimilis* has given strong evidence that these sibling species do not interbreed. Similar studies on *D. paulistorum* do not rule out the possibility of some gene exchange, at present or in a recent past, between the semispecies (*18*). *Drosophila paulistorum* is a superspecies which still conserves a common, although deeply fissured, gene pool.

No other superspecies quite like *D. paulistorum* have yet been discovered in *Drosophila.* This is not surprising in view of the tendency of the speciation process in these flies to occur with little or no visible differentiation in externally visible characteristics. The remarkable cluster of forms that constitute the *D. mesophragmatica* complex (*28*) come perhaps closest to the status of a superspecies composed of semispecies. Superspecies and semispecies are known in birds, mammals, butterflies, mollusks, and planarians (*1*), but few of them are suitable for experimental study. They are known also in the plant kingdom (*29*).

Magnitude of Genetic Differences between Species

Classical theories of population structure grossly underestimated the amount of genetic variability. The usual assumption was that the bulk of individuals of a sexual, diploid, and outbreeding species (such as man or *Drosophila*) are homozygous for the same wild-type gene alleles at a great majority of gene loci. Consonant with this, related species were surmised to differ in only few genes; several "lucky" mutations, possibly a single one, could give rise to a new species. Doubt was cast on these beliefs by the discovery of vast amounts of variability concealed in "normal" individuals and populations. This variability consists chiefly of recessive gene alleles and gene complexes, and in *Drosophila* and some other forms, of inversions of blocks of genes in some chromosomes (*4*). Gradually it became clear that no two individuals in a sexual outbreeding species are at all likely to be genetically identical. However, it was only through analyses of enzyme and other protein polymorphisms and monomorphisms by electrophoretic techniques that it became possible to obtain even rough estimates of the proportions of genes that are represented by similar or distinct alleles in individuals of the same or different species (*30*).

About ten species of *Drosophila* have been studied more or less extensively for protein polymorphisms. Between 25 and 70 percent of the gene loci proved to be polymorphic in natural populations, that is, represented by two or more alleles with appreciable frequencies. An individual fly in these populations is estimated to be heterozygous for between 8 and 28 percent of its genes (*30, 31*). Estimates within the above ranges have been obtained also for animals other than *Drosophila*, man included. To what extent the differences between the estimates recorded for different species are real, and the genes coding for the proteins studied are unbiased samples of all genes, are open questions. However, let us take the lowest of the above estimates, and accept the figure 100,000 as the number of gene loci in *Drosophila.* Some 25,000 genes are then polymorphic in a *Drosophila* population, and an average fly is heterozygous for some 8,000 genes. Genetic variability of this magnitude would have seemed unbelievable to most geneticists even a decade ago!

A study of the proportions of proteins that are electrophoretically similar and different in species of *Drosophila* has been pioneered by Hubby and Throckmorton (*32*). These workers examined nine triplets of species, each triplet including two sibling species and one closely related but easily distinguishable species. The different triplets belonged to different species groups, or to different subgenera of *Drosophila.* Thus, they had three levels of structural, and presumably also genetic, differentiation. The sibling species had on the average only 50 percent of their proteins in common, the percentages ranging from 23 to 86 for different pairs. The two siblings and the related nonsibling shared only 11.6 percent of the proteins on the average, and members of different triplets were even less similar. By contrast, some related but morphologically distinguishable Hawaiian species of *Drosophila* proved to be remarkably similar in their electrophoretically diagnosed proteins (*33*).

A more detailed study has been made of the sibling species of the *willistoni* group (*34*). Wherever possible, samples from different parts of the geographic area of each species were examined. Hubby and Throckmorton's findings (*32*) were confirmed—individuals of different sibling species differ on the average in somewhat more than one half of their gene loci. Perhaps even more impressive are the percentages of the loci that are diagnostic for the species, that is, that permit identification of the species in single individuals by their protein variants (*20*). The percentages of the diagnostic loci are shown in Table 1. Even *D. paulistorum* and *D. pavlovskiana*, siblings so close that they were originally regarded semispecies of a superspecies, can be diagnosed by an estimated 14 percent of their genes.

Very promising beginnings have been made in estimation of the magnitudes of species differences by means of their DNA (*35*) or their DNA-RNA "hybridizations." The complementary strands of the DNA chain molecules can be separated by heating, and the separate strands can then reanneal when the temperature is lowered. The DNA's of different species undergo the reannealing more slowly or not at all, depending upon the degree of the resemblance of their nucleotide sequences. Similar in principle is the hybridization, or annealing, of separated DNA strands with RNA transcribed from them. Both techniques have been used to compare the closely related species *D. melanogaster* and *D. simulans*, and also *D. funebris* which belongs to a subgenus different from the first two. The DNA's of *D. melanogaster* and *D. simulans* are estimated to contain about 80 percent of the nucleotide sequences in common, while the DNA of *D. funebris* has only about 25 percent of sequences similar to the other two species. The hybridization of the DNA of *D. melanogaster* with RNA of *D. simulans*, or vice versa, is only 40 to 50 percent as effective as that of DNA and RNA of the same species; with *D. funebris* the effectiveness drops as low as 10 percent (*36*). It would be premature to use these figures as estimates of the proportions of similar and dissimilar genes in these species. The figures reveal mainly the so-called repeated sequences, that is, the genes that are represented numerous times within a single gene complement. Nonetheless the great extent of the interspecific differences is most interesting.

Table 1. Percentages of gene loci coding for electrophoretically distinguishable protein variants that are diagnostic for any two sibling species of the *Drosophila willistoni* group with a probability greater than .99 for each locus (*20*).

Species	*tropicalis*	*equinoxialis*	*paulistorum*	*pavlovskiana*	*insularis*
D. willistoni	17.9	21.4	25.0	25.0	32.1
D. tropicalis		21.4	35.7	28.6	28.6
D. equinoxialis			14.3	25.0	28.6
D. paulistorum				14.3	32.1
D. pavlovskiana					32.1

Chromosomal Differences

From the above data it cannot be validly inferred that species in general, or that species of *Drosophila* in particular, always differ in numerous genes. Speciation can occur in more than one way. Biologists who are too fond of simplicity and homogeneity may be chagrined by the "inventiveness" of the evolutionary process.

The availability in *Drosophila* of giant polytene chromosomes facilitates the analysis of the variations in the gene arrangements within and between species. It has been known for several decades that natural populations of many species are polymorphic for variant chromosome structures, owing mainly to the occurrence of inversions of blocks of genes. Yet some species are chromosomally monomorphic [see (*4*)]. Species, sibling as well as morphologically distinct species, may have the genetic materials in their chromosomes more or less radically rearranged. Individuals of the sibling species *D. pseudoobscura* and *D. persimilis* differ in at least two, usually more, inversions. The six siblings of the *willistoni* group can be simply and conclusively identified by inspection of their chromosomes in the larval salivary gland cells. The number and kinds of the chromosomal reconstructions have not been precisely identified. *Drosophila pseudoobscura* and *D. willistoni* with their siblings belong to different sections of the same subgenus (*Sophophora*). The gene arrangements in their chromosomes have diverged so greatly that not only can the species be discriminated at a glance, but the corresponding (homologous) chromosomes can no longer be recognized (further examples in *4*, *37*, *38*).

The discovery of homosequential species came as a considerable surprise (*12*, *39*). These are species that have identical gene arrangements in their chromosomes, as inferred from the banding patterns in the polytene chromosomes, in cells of the larval salivary glands. Carson (*21*) lists ten groups of Hawaiian endemics, with two to five homosequential species per group. He emphasizes that these are not siblings but morphologically easily distinguishable forms, some of them even strikingly different in outward appearance. The conclusion is inevitable that genetic divergence and speciation may occur without rearrangement of the genetic materials in the chromosomes, although more often the two processes go hand in hand. It may be noted that homosequential species are not confined to Hawaii, since at least one example of continental homosequential species has been recorded (*40*).

Founder Principle and Neospecies

The usual, by now orthodox, view among evolutionists has been that species formation occurs by slow genetic divergence, and subsequent reproductive isolation, of geographically separated and differentially adapted races or subspecies (Darwin's "varieties"). Sudden emergence of new species by allopolyploidy is an exception, irrelevant to *Drosophila* and most bisexual animals. In several brilliantly argued contributions (*12*, *21*, *41*, for example), Carson advances a novel and unorthodox view—speciation may occur rapidly, and a neospecies of *Drosophila* may, without prior adaptive divergence, emerge within relatively few generations. The idea stems from Mayr's founder principle (*1*, *7*, *42*), which is in turn a special case of Wright's random genetic drift (*4*).

The founder principle is "establishment of a new population by a few original founders (in an extreme case, by a single fertilized female) that carry only a small fraction of the total genetic variation of the parental population" (*1*). Founder events are inevitably followed by inbreeding for one or several generations. The populations descended from the founders are then restructured by natural selection, which operates on a changed gene pool and usually in an altered environment. This theoretical scheme was verified in experiments with chromosomally polymorphic populations of *D. pseudoobscura* (*43*). Natural selection in experimental populations derived from small numbers of founders resulted in a greater variety of outcomes than in comparable populations descended from numerous founders. It should be noted that, although the genetic variability among the descendants of a single pair of founders is reduced compared to the population from which the founders came, it is by no means absent. Experiments on several species of *Drosophila* have shown that recombination of genes in a single pair of chromosomes drawn at random from a natural population can give rise to considerable genetic variability (*44*).

Carson postulates that founder events

Table 2. Observed matings between selected and unselected strains of *Drosophila paulistorum*. (LU, Llanos unselected; LS, Llanos selected; OU, Orinocan unselected; OS, Orinocan selected.)

Strains		Matings				Isolation coefficient
A	B	A♀ × A♂	A♀ × B♂	B♀ × A♂	B♀ × B♂	
LU	OU	61	44	51	65	0.14 ± 0.07
LS	OS	52	4	4	41	0.67 ± 0.07
LS	OS	45	7	3	54	0.82 ± 0.05
LS	OS	46	6	8	49	0.74 ± 0.06
LS	LU	44	64	29	66	0.08 ± 0.07
OS	OU	24	26	25	38	0.10 ± 0.09

must have played a key role in the spectacular proliferation of species of drosophilid flies on the Hawaiian archipelago. These volcanic islands were never connected with any continent, and most of them not with each other. The oldest island inhabited by drosophilids (Kauai) is some 5.6 million years old, the youngest (Hawaii) is only 0.7 million years old, while others (Oahu, Maui) are intermediate in age. Their drosophilid fauna, about 500 species, is descended from probably two, or even a single species, introduced by accidental long-distance transport across the ocean. A great majority of the species are endemic not only to the archipelago but to single islands (or adjacent islands which were connected in geologically recent past). With the aid of cytogenetic, morphological, and distributional studies, it is possible to establish with a high degree of probability which species on one island, particularly a geologically younger one, have descended from ancestors similar or identical with species on the older islands. The youngest and largest island, Hawaii, has a particularly interesting array of 11 groups of 23 species, the nearest relatives of which are found mostly on the next youngest island, Maui, and only one each on Oahu and Kauai.

Carson infers that each species is descended from a single gravid female that arrived from the donor island. What is remarkable is that these single founders gave rise to new species, rather than simply to new colonies of the old donor species. Carson argues that the genetic upheavals that result from the initial inbreeding, followed by rapid expansions of the newly founded populations, are propitious for the origin of reproductively isolated neospecies. Reproductive isolation arises, according to his view, as a chance concomitant of the genetic upheaval that follows the founding event. In his words: "The key genetic shifts leading to the crucial species differences may be non-adaptive. I suggest that they may precede, in time, an adaptive phase wherein a large genetically variable population is exposed to the usual and well known forces of natural selection" (*21*).

This is a radical departure from the orthodox view. What is the biological function of speciation? The most reasonable interpretation seemed to be that speciation makes the adaptive divergence of evolving populations irreversible. Or to put it differently—reproductive isolation safeguards the adaptive gene systems that have evolved in differentially adapted species from disruption owing to gene exchange. According to Carson's scheme, reproductive isolation and speciation precede differential adaptedness. If so, speciation would seem to be devoid of biological function, until a differential adaptedness arises following the speciation. This is not merely a new form of the old dispute, whether reproductive isolation is simply a by-product of adaptive divergence, or an ad hoc contrivance built by natural selection (*1*, *4*). The issue is whether reproductively isolated species remain, at least for a time, adaptively equivalent in similar environments. Be it noted that Carson does not claim universality for his speciation scheme: ". . . where a widespread continental species becomes broken up into subspecies, the result is frequently a gradual population change involving both adaptation and speciation" (*41*). It may well be that two fairly contrastive methods of speciation occur among drosophilid flies, as well as among other organisms. If so, only future research can give a measure of the incidence and importance of these methods. One would like to know, for example, whether the proportions of genes involved in differences among neospecies are comparable to those found between "good" species.

Origin of an Incipient Neospecies in the Laboratory

The origin of neospecies through the process postulated by Carson may conceivably be observed in the laboratory. Such an event may have taken place in my laboratory sometime between 1958 and 1963 in a strain of *D. paulistorum*. This strain is descended from a single inseminated female captured in the Llanos of Colombia in March 1958. As described above, the superspecies *D. paulistorum* consists of five semispecies. The semispecies show a strong, although incomplete, sexual isolation, complete sterility of hybrid males, and sometimes geographic isolation. When tested in 1958, the Llanos strain gave fertile hybrids with strains of the Orinocan semispecies, and was accordingly considered to belong to that semispecies. Yet from 1963 onward, it has produced sterile male hybrids when crossed to Orinocan. The emergence of the hybrid sterility was not, however, accompanied by ethological isolation. The changed Llanos strain mates freely with Orinocan, as though it still belongs to that semispecies (*45*).

In experiments started in 1966, I and Pavlovsky have endeavored to superimpose an ethological isolation on the existing hybrid sterility by artificial selection (*46*). Two recessive mutants, rough eye in the Llanos and orange eye in an Orinocan strain, are being used as markers. In every generation rough-eyed Llanos and orange-eyed Orinocan females are exposed to mixtures of males of both kinds. Matings of likes (that is, rough crossed with rough and orange crossed with orange) produce progenies showing the respective mutant traits. Matings of unlikes produce hybrid flies with nonmutant (wild type) characteristics. The hybrids are destroyed, and the selection is carried forward by again exposing rough or orange females to both kinds of males. Thus far the selection has been carried for exactly 100 generations. The Llanos and Orinocan strains no longer mate at random (Table 2).

It can be seen that the unselected (U) Llanos (L) and Orinocan (O) strains mate among themselves almost as frequently as they do with each other (line 1 in Table 2). After 50, 58, and 90 generations of selection (lines 2 to 4 in Table 2) matings within the selected (S) strains are decidedly more frequent than between them. A convenient measure of the departure from randomness in mating is the isolation coefficient devised by Professor Howard Levene (of Columbia University); this coefficient is zero if matings are at random, and unity if the ethological isolation is complete. The coefficients achieved by selection (0.67 to 0.82) are of about the same magnitude as those observed between the more closely related natural semispecies. The selected Llanos and Orinocan strains continue however to mate at random with the respective unselected controls without the mutant gene markers (lines 5 and 6, Table 2).

That the selected Llanos strain should be considered at least an incipient neospecies is warranted. The nature of the event responsible for the initiation of the hybrid sterility between Llanos and Orinocan some 20 years ago remains a problem, however. One possibility is that a change took place in the population of intracellular symbionts, which seem to be present in all strains of the superspecies *D. paulistorum* (*47, 48*). A second possibility has to do with the geographic derivation of the Llanos strain. This strain comes from a marginal locality where the distribution area of the Orinocan semispecies abuts the mountain range of the Andes. According to Carson, geographically marginal populations may be subject to changes of the same sort as island populations derived from single founders. A third possibility is that the Llanos strain was a form intermediate, and possibly even hybrid, between the Orinocan and Interior semispecies. Interior (which was discovered after the changes in the Llanos strain) is a near relative of Orinocan, and the changed Llanos gives fertile male hybrids with Interior strains. There is obviously no way to tell whether Llanos would have given fertile hybrids with Interior as well as with Orinocan in 1958. Anyway, what happened was not simply a conversion of Interior to Orinocan; there is a pronounced ethological isolation between these semispecies, and not between the unselected Llanos and the Orinocan.

Conclusion

Anything that can be said about a new, or recently renewed, field risks being superseded or negated by further discoveries. As the situation appears to be now, there is one consideration which seems unlikely to be changed: there is not a single kind but there are several kinds of species and of processes of speciation in *Drosophila* and, of course, even more in the living world at large. As a category of classification, species was and is being applied to all organisms, and this has led to futile search for universal biological properties of all species. What is actually found is a remarkable variety of different kinds of species. Even confining our attention to sexually reproducing and outbreeding forms, we find more or less monolithic "good" species, superspecies, and semispecies. Finally, it begins to look as if reproductive isolation may sometimes follow and at other times precede the adaptive divergence of gene pools of populations.

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