Chromosome Tracers of the Origin of Species

Some Hawaiian *Drosophila* species have arisen from single founder individuals in less than a million years.

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The most spectacular aspect of organic evolution is the origin of adaptations (*1*). These arise primarily as the result of genetic changes which are incorporated into populations through the action of natural selection on a variable and freely recombining pool of genes. The population genetics of such evolutionary change has been well understood since the theoretical work of the 1930's (*2*). The origin of certain adaptations on the microevolutionary level (for example, protectively colored moths in industrially blackened areas) has been closely analyzed (*3*).

Adaptive evolution is basically a phyletic process—that is, it occurs during succeeding generations within naturally interbreeding populations of a single biological species. To put it another way, this kind of evolutionary change can occur without any multiplication of reproductively isolated population units, or species. Both phyletic change and speciation are evolutionary processes—that is, both involve descent with change. Whereas phyletic evolution has yielded to elegant mathematical and experimental analysis, the speciation process has been generally refractory to studies of comparable precision. In this sense, the origin of species is a major unsolved problem of evolutionary biology. The present article provides a new approach which may help in its solution.

Speciation is almost exclusively a geographical process (*4*). Populations of existing species are entities distributed in space and time; somehow, such a species population becomes split into subpopulations (subspecies). In time, one or more of these latter may emerge as species.

The difficulties of studying the dynamics of geographic speciation are manifold. In the first place, it is a slow process by human standards. The formation of a species in the usual continental situation probably takes thousands or hundreds of thousands of years or more, even in organisms which have a relatively rapid generation time. Furthermore, the geographical relationships of most organisms are extraordinarily complex. Species populations which are in the crucial initial stages of evolutionary divergence may be very difficult to recognize. Many species have worldwide or at least continental distributions; this makes it difficult to decide which portion of the species, if any, deserves study from the point of view of the speciation process. Then again, continental or widespread species often have enormous populations, through which gene flow may be active. Such flow may inhibit the very speciation processes the evolutionist wishes to concentrate on. If endemic continental species with small, semi-isolated populations are selected for study, it is often difficult or impossible to tell whether this represents an incipient species or whether it is an ancient isolation with secondary intergradation. Continents and their biotas are thus frequently too complex geographically, historically, and ecologically to permit accurate inferences concerning the process of the origin of species. Islands, especially oceanic islands, have simpler conditions.

An oceanic island is one that is thrown up in the vastness of one of the earth's great oceans. The volcanic action which is frequently responsible produces at first a fiery, sterile mass. When cooling occurs, life starts to move in. Mostly it comes by chance, through long-distance dispersal, especially if the islands concerned—like the Hawaiian Islands, for example—are thousands of miles from any other land mass. Those lucky few propagules that may reach a new volcanic island and establish colonies set in motion forces which provide a new evolutionary beginning for the group concerned. Even though the propagule that arrives may already have millions of years of evolution behind it, the isolation from its ancestral relatives and contemporaries, coupled with the new, raw, and often difficult ecological conditions it faces, provides a renewed evolutionary opportunity. The results often strike the continental biologist as bizarre, "explosive," or otherwise extraordinary when measured against experience with life on the continents.

Contemplating the fauna of the oceanic Galápagos Islands, Darwin wrote, with characteristic understatement, "the inhabitants of these islands are eminently curious." As everyone knows, his observations of the simplified conditions existing there led him to a train of thought which catapulted evolutionary thinking into the center of biological attention, a position which it still holds today.

The Hawaiian Islands are in many ways uniquely suited for the study of the process of speciation. They are by far the most isolated oceanic islands in the world. The archipelago is 2000 miles (3200 kilometers) from any continent and lies in the warm tropical region of the Pacific Ocean. The vast volcanoes, of which the islands are the emergent tops, rise to great heights; the summit of Mauna Kea is more than 4200 meters above sea level and acquires a snow mantle each winter. The slopes of the islands erode rapidly under the heavy tropical rains. The older volcanic domes are dissected into deep valleys, separated by sharp ridges. Most of the rain falls on the windward slopes, leaving the lee sides desert-like. These features combine to produce extraordinarily diverse habitats: there are windswept alpine meadows, rain forests, and blisteringly hot southwestern lowland slopes.

Terrestrial life came to Hawaii by chance from all directions; descendants of these few ancestors have populated the islands with a unique biota. Chance, it appears, not only affected what organisms reached the older islands 5 million or more years ago but also played a role in the spread of life within the

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archipelago from the older islands to the younger. Although this evolution has produced some remarkable biological innovations and adaptations, the paramount feature is the enormous number of species of flowering plants, ferns, terrestrial invertebrates, and, especially, insects (*5*).

The genus *Drosophila* as it exists on the continents includes hundreds of species of small flies most of which are adapted to humid environments where they breed on decaying or fermenting vegetation. They are easy to handle in the laboratory, and certain species have become prime objects for the study of evolutionary and population genetics (*6*). From the Hawaiian Islands, considerably smaller in area than the state of Massachusetts, more than 250 species of the genus *Drosophila* have been described (*7*). All but about 12 have evolved in Hawaii and are found nowhere else. This number is approximately one-fourth of the species of the genus known in the entire world. It is probable, furthermore, that many *Drosophila* species in Hawaii are yet to be described. The islands also have a large number of species of Drosophilidae belonging to genera closely related to *Drosophila* (for example, *Scaptomyza*). Clearly, Hawaii has one of the greatest concentrations of this family of flies in the world.

The Hawaiian Islands appear to be geologically very recent (*8*). Thus, potassium-argon measurements (*9*) indicate that the oldest lava flows, on the northernmost island, Kauai (Fig. 1), are approximately 5.6 million years old, an age that places them in the late Pliocene. On the other hand, the island of Hawaii appears to have been formed very recently indeed. Thus, lava flows on the Kohala volcano yield both potassium-argon and paleomagnetic data which indicate that the mountain is no older than 700,000 years (late Pleistocene). The four southernmost volcanoes, two of which are currently active, are even younger than this. The adjacent island of Maui, separated from Hawaii by a 30-mile-wide channel (Fig. 1), is also of Pleistocene age; its lava flows give ages from 1.3 to 1.5 million years. Thus, Maui is considerably older than Hawaii. The channel between the two islands (Alenuihaha Channel) is 1950 meters deep, and it appears that Maui was never connected to Hawaii by a land bridge. On the other hand, there is strong evidence that Pleistocene land bridges once linked the present islands of Maui, Molokai, and Lanai (Fig. 1).

In short, it is clear that the island of Hawaii is the youngest in the archipelago. In this article I focus attention on the origin of certain of the *Drosophila* species endemic to this island. Chromosomal data make it possible to trace the precise ancestry of a number of these species from certain Maui populations.

Chromosomes of Hawaiian Drosophila

Extensive accounts of the evolutionary biology of Hawaiian *Drosophila* have been recently published (*10–12*). Among these are descriptions of nearly a hundred species of large flies forming a clear subgroup belonging to the genus *Drosophila*. Because they are characterized by patterns of dark spots on the wings, these species have been informally referred to as the "picture-winged" flies (see cover). All so far examined have a metaphase chromosome group of $2n = 12$ (*13*). The five major polytene chromosomes of 69 of these species have been mapped in terms of a set of arbitrary Standard band sequences—namely, those found in the species *D. grimshawi* from Auwahi, Maui. Banding comparisons of unknowns with the Standard have been facilitated by the use of a binocular drawing tube (*14*). This device permits comparison of the sequence of a chromosome of unknown banding order under the microscope directly at table level with a photographic cutout of a known Standard sequence (Fig. 2).

Except for one case of an apparent deletion, all microscopically observable polytene chromosome mutations in these species are due to changes in gene order (paracentric inversions). One hundred and fifteen inversions have been fixed among the 69 species of picture-winged flies (*10*). Each inversion has been designated by a separate lowercase letter after the chromosome number; the alphabet has been used several times with numerical superscripts—for example, a^2, b^2, and so on. By means of the method of Wasserman (*15*), a chromosomal phylogeny based on inversion-sharing has been prepared. Such phylogenies contain no intrinsic information about the direction of evolution. That is, it is possible to start at any point and derive all the other sequences in a stepwise fashion. The designation of a starting point, which converts such a relationship diagram into a phylogeny showing direction of evolution, must come from information outside the data on chromosomal sequences.

Relevant outside geographical and geological information exists in this case. First, it seems clear that the *Drosophila* fauna of the Hawaiian Islands is derived from the mainland, rather than vice versa. In this connection, one of the picture-winged subgroups (the *D. primaeva* subgroup, known so far only from Kauai) shows a sequence in the relatively conservative

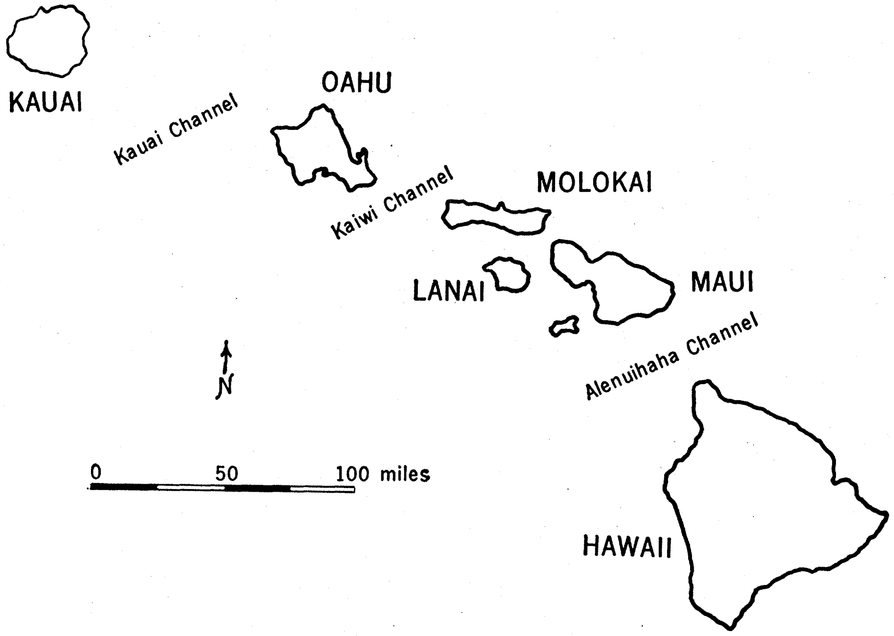

Fig. 1. The six major islands of the Hawaiian Archipelago.

Table 1. Comparison of chromosomal formulas between certain *Drosophila* species from the island of Hawaii and their Maui counterparts. Lowercase letters refer to specific fixed inversions.

Island	Species	Chromosomal formula				
		Set No. 1				
Hawaii	*D. silvestris*	Xijkopqrst	2	3d	4b	5
	D. heteroneura	Xijkopqrst	2	3d	4b	5
Maui	*D. planitibia*	Xijkopqrst	2	3d	4b	5
		Set No. 2				
Hawaii	*D. ciliaticrus*	Xg	2	3o	4	5
	D. engyochracea	Xg	2	3	4l	5
	D. murphyi	Xg	2	3/3o	4	5
Maui	*D. orphnopeza*	X	2	3/3o	4	5
	D. balioptera	Xg	2	3	4	5
	D. orthofascia	Xg	2	3n	4	5
		Set No. 3				
Hawaii	*D. setosimentum*	Xikouvwxym^2	2cdl	3fjkl	4bopqb^{2}c^{2}d^{2}e^{2}f^{2}n^{2}o^2	5f
	D. ochrobasis	Xikouvwxym^2	2cdk	3fjk	4bopqb^2	5f
Maui	*D. adiastola*	Xikouvwxy	2cd	3fjk	4bopq	5f

chromosome 5, which is the closest arrangement among any of the island flies to the homologous chromosome in certain Palearctic-Nearctic mainland species of the subgenus *Drosophila* (*16*). This fact places the *D. primaeva* subgroup at the base of the Hawaiian chromosome phylogeny. Further evidence on the direction of evolution comes from facts pertaining to the increasing geological youth of the Hawaiian islands as one proceeds southeastward from the northernmost major island, Kauai. These facts all serve to focus on, and to underscore, the newness—indeed, the present terminal nature in space and time—of the fauna of the island of Hawaii itself.

Origin of Species on the Island of Hawaii

Of approximately 21 species of picture-winged flies in collections from the island of Hawaii (Fig. 1), 17 have been analyzed for polytene chromosome sequences. All 17 are highly distinctive species. They are endemic to this island, being found nowhere else in the world, not even on the island of Maui, only 30 miles away. Although much smaller in size than Hawaii, the Maui complex is richer in species of this group. Of approximately 41 species known in collections, 30 have been analyzed chromosomally.

So far, all of the species from the island of Hawaii tested by hybridization techniques appear to be reproductively isolated from all other species. Thus, in studies with laboratory strains, no fertile hybrids have been obtained between any of the Hawaii species or between these species and their Maui relatives. The data, however, are not extensive (*17*).

Seven of the 17 species from Hawaii are of particular interest. The chromosomal formulas for these species relative to the Standard (X 2 3 4 5) are given in Table 1. The method of notation may be illustrated by the formula for *Drosophila ciliaticrus*, which differs from the Standard by one inversion in the X chromosome (Xg) and one in chromosome 3 (3o). Illustrations showing the breakpoints of these inversions have been published (*10*). Chromosomes 2, 4, and 5 of this species have the Standard gene order.

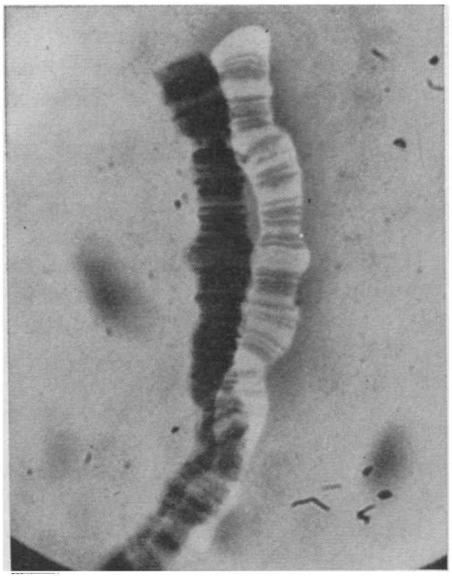

Fig. 2. Photomicrograph of a chromosome end (left, darker object) viewed simultaneously with a cutout of a photograph (right, lighter object) of a portion of the homologous chromosome of the Standard, *Drosophila grimshawi*. The image of the chromosome end is juxtaposed to the cutout at table level by a binocular drawing tube. This facilitates matching of the banding orders.

The seven Hawaii species listed in Table 1 fall into three sets; each set is related to one or more species known from Maui. The first set of two species, *Drosophila silvestris* and *D. heteroneura*, are homosequential—that is, they have identical arrays of fixed inversion sequences relative to the Standard. In addition, the two species are homosequential with respect to a third species, *D. planitibia* from Maui (Table 1).

Homosequential species are a striking feature of Hawaiian *Drosophila*. Their homosequentiality not only underscores their very great basic similarity but also makes clear the fact that much evolutionary chromosome change occurs at the submicroscopic or molecular level (*18*). In addition to *D. planitibia* and its two Hawaii relatives, 11 more such homosequential sets have so far been recognized from all the islands, involving 36 species in all (*12*). Members of a homosequential set often vary so much that studies of morphology, genitalia, and behavior sometimes fail to suggest that they should be grouped together.

In the present case, however, the significant fact is that, of the 13 species which fall into the *Drosophila planitibia* subgroup of the picture-winged flies (*10*), only *D. planitibia, D. silvestris,* and *D. heteroneura* have the key inversion Xr. This is one of the nine inversions by which the X chromosome of these species differs from Standard. Thus, of all the possible candidates for an ancestor of the two Hawaii members, only *D. planitibia* of Maui fulfills the requirements chromosomally.

Accordingly, it is concluded not only that the ancestor of the Hawaii species was derived from Maui but also that it may be specifically traced chromosomally to a population ancestral to the present-day *Drosophila planitibia.* A founder which crossed the Alenuihaha Channel following the raising of the island of Hawaii above sea level is inferred (Fig. 3). The chromosomal formula for this putative ancestor is given across the arrow in Fig. 3.

A founder for the *Drosophila murphyi* complex of three species (Table 1, set No. 2) can also be inferred; it has some special properties of interest. All three species from Hawaii show the inversion Xg. *Drosophila ciliaticrus* has, in addition, the fixed condition 3o, but *D. murphyi* populations are polymorphic, carrying both the Standard 3 and 3o. All expected karyotypes (the homozygotes 3/3 and 3o/3o as well as the heterozygote 3/3o) have been found within present-day populations of this latter species. *Drosophila engyochracea* does not have 3o but has a new fixed inversion (4 l) which it does not share with any other known species.

No single Maui species is known which combines both the fixed Xg and the polymorphic 3/3o karyotypes. On the other hand, two species collectively fulfill these conditions. Thus, *Drosophila balioptera* has Xg only, whereas *D. orphnopeza* populations, like those of *D. murphyi,* are polymorphic for 3/3o. This species, however, has the Standard X chromosome. Accordingly, it is inferred that an ancestral population once existed on Maui which was polymorphic for both X/Xg and 3/3o. A migrant from this population, carrying Xg in the fixed state and the heterozygous condition 3/3o, reached the island of Hawaii as the founder which subsequently gave rise to the *D. murphyi* cluster of species. Present-day *D. murphyi* appears to have the chromosomal formula closest to that of this putative ancestor (Table 1 and Fig. 4). In the process of descent, it appears that *D. ciliaticrus* has fixed 3o, whereas *D. engyochracea* has refixed Standard 3. As in the case of the *D. planitibia* subgroup, the key inversions which serve as tracers are unknown in species other than those listed and, most significantly, are not found among any of the many species known from Oahu or Kauai. They appear to be specific "Maui-Hawaii" markers, having apparently arisen by mutation in a past population on the Maui complex.

The third case involves the two species *Drosophila setosimentum* and *D. ochrobasis* of Hawaii, which relate to *D. adiastola* of the Maui complex (Table 1). Each of the Hawaii species has a basic group of 18 inversions in common with *D. adiastola.* On the other hand, the two Hawaii species have certain new inversions (Xm^2, 3l, and $4b^2$). In addition, each has certain inversions of its own which are not found in the other. A striking feature is the accumulation of a large series of 4th chromosome inversions in *D. setosimentum.* This process appears to be continuing, as *D. setosimentum* shows extensive intraspecific 4th chromosome polymorphism in addition to the fixed inversions shown in Table 1 (*10*).

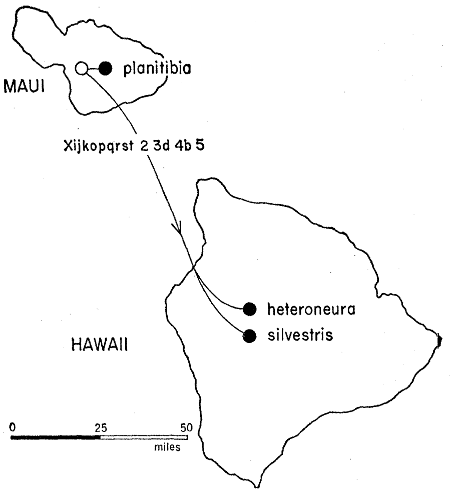

Fig. 3. A postulated interisland species founder going from Maui to Hawaii during the late Pleistocene. The open circle represents a population ancestral to the three present-day species *Drosophila planitibia, D. heteroneura,* and *D. silvestris* (solid circles). The inferred chromosomal formula of the interisland founder is superimposed on the arrow.

As in the other two cases of Table 1, it is concluded that the two modern Hawaii species of this complex are descended from a founder stemming from a population directly ancestral to present-day *Drosophila adiastola* and homosequential with it (Fig. 4).

Of the five members of the *Drosophila adiastola* subgroup of flies of the Maui complex, two others are homosequential with *D. adiastola* and appear to mark equally well the ancestral lineage of the two Hawaii species. One of these, *D. cilifera,* is endemic to Molokai, and the other, *D. peniculipedis* of Maui, has a peculiar constriction in chromosome 4 which is not found in the Hawaii species. In any event, the founder is likely to have been derived from a population ancestral to these three homosequential species, rather than from any one modern species.

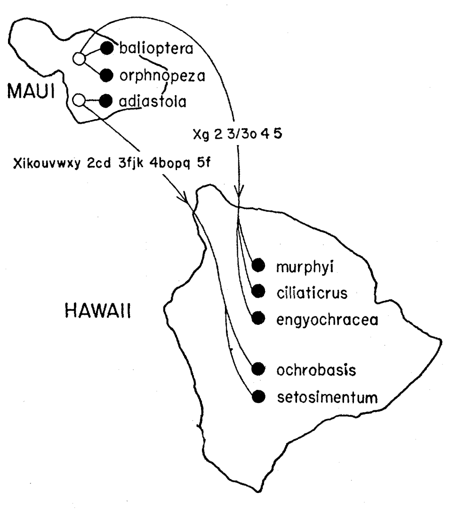

Fig. 4. Two additional interisland species founders going from Maui to Hawaii (see legend to Fig. 3, and text).

The three cited founder events are not isolated cases; there are at least three other known instances of one or more Hawaii species that has its closest chromosomal relative on Maui (*10*). They are less diagrammatic than the cases discussed above, however, because, in these other instances, similar homosequential species occur on Oahu or Kauai, thus founders could have come to the island of Hawaii directly from one of those islands, bypassing Maui.

The Founder Event and Speciation

The island of Hawaii, which is the youngest in the archipelago, has apparently received much of its picture-winged *Drosophila* fauna from the adjacent island of Maui. In three especially clear instances the founders may be traced chromosomally to Maui only, and their precise inversion formulas may be inferred. The species of the island of Hawaii are endemic to that island, and, since the island appears to be no more than 700,000 years old, the evolutionary events which produced the species must have consumed less time than that. Thus, these species must have evolved less than three-quarters of a million years ago on the island of Hawaii. Such precise statements can rarely be made about evolutionary events.

There is no evidence of repeated simple colonizations of the island of Hawaii. No subspecies of species from Maui or any other island have as yet been found there, at least in the group under discussion. This suggests that the founder event is an exceedingly rare one, and that the break between the donor population and the new colony on the invaded island is a deep one biologically.

One of the most striking features of this situation is the fact that speciation invariably has followed the founder event. This suggests further that the founder event may be accomplished by a single propagule, probably a single fertilized female.

In Figs. 3 and 4, three founder events have been postulated as being responsible for the origin of seven species. In each case the law of parsimony has been invoked and it has been assumed that, for instance, only a single founder was ancestral to the clusters of two (or, in one case, three) species which occur on the island of Hawaii. On the other hand, the "one founder, one new species" view might be defended. It appears, however, that if such double colonizations from a chromosomally identical stock did occur, the likely result would be subspecies of some Maui species, not new unique species, such as are observed.

As was proposed above, evolution seems very often to display two major processes: an adaptation (fitness for a specific function in a specific environment) appears to be made by intraspecific phyletic change, whereas species result from a multiplicative process whereby populations become divided and isolated. The situation on oceanic islands, as revealed in the data given here, suggests a clarifying separation, in time and in process, of these two aspects of evolution. Thus, the hypothesis may be made that a speciation phase precedes an adaptive phase. The speciation episode appears to be characterized by the successful colonization of a relatively open ecological habitat by a single founder female. This event would be expected to have several important genetic consequences. First, a maximization of the phenomenon of random genetic drift would be accomplished as the new colony is formed. Second, early success of the new colony in a locally permissive habitat might result in a population flush. Certain new genetic recombinants might be multiplied and preserved by chance in the demes which survive the inevitable population crash.

The resulting interdeme selection and related processes, it is argued, might well lead to the appearance of a genetic gap between the new colony and its progenitor. This gap might be recognized as the equivalent of speciation (*19*). In my view, this might be accomplished in a relatively small number of generations. If this is the case, then the synthesis of species under controlled conditions might indeed be accomplished in the laboratory or field plot more easily than has been previously thought. Indeed, a striking report of a suggestive case has appeared recently (*20*).

When the gene pool of a population is being rigorously shaken up by chance events of the kind discussed above, speciation is not likely to be accompanied by adaptive evolution. It is suggested that, where species founders play a role, as in these terrestrial populations of oceanic islands, the adaptive evolutionary phase is likely to occur only after the chance events leading to speciation are essentially complete. Thus, the gene pool of the new species undergoes new phyletic evolution in isolation from its ancestors and contemporaries. Since genetic drift and the founder effect undoubtedly provide a genetic revolution (*21*), the species is faced with the alternatives of extinction or the welding of a somewhat discordant gene pool into something ecologically workable. The result is seen in the somewhat bizarre yet generally well-adapted products of insular speciation.

The separation of phyletic evolution from speciation, as called for in the foregoing hypothesis, would not, of course, be expected to apply to all patterns of speciation. For example, where a widespread continental species becomes broken up into subspecies, the result is frequently a gradual population change involving both adaptation and speciation. Thus, in these cases, it is probable that speciation and adaptation are synchronic population processes. Only where the founder effect is prominent may the features of the two processes be clearly seen as essentially separate evolutionary phases, one with and one without a large element of chance.

Summary

Organic evolution produces species and adaptations. Data from terrestrial populations existing on oceanic islands suggest that the processes whereby species and adaptations arise are different and are sometimes separated in time. Thus, in *Drosophila* from the island of Hawaii, speciation appears to follow the establishment of a new island population from a single founder individual from a nearby island. In these cases, it is hypothesized, speciation is characterized by isolation, random genetic drift, and the abrupt, nonadaptive changes in the gene pool which would be expected to immediately follow the founder event. The process is aided by interdeme selection. Adaptations, which follow in time, are forged genetically by the well-known intrademic processes of mutation, recombination, and selection.

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