Hawaiian Drosophila: Young Islands, Old Flies

Molecular evidence on Hawaiian drosophilines indicates that they colonized the 6-million-year-old islands more than 40 million years ago

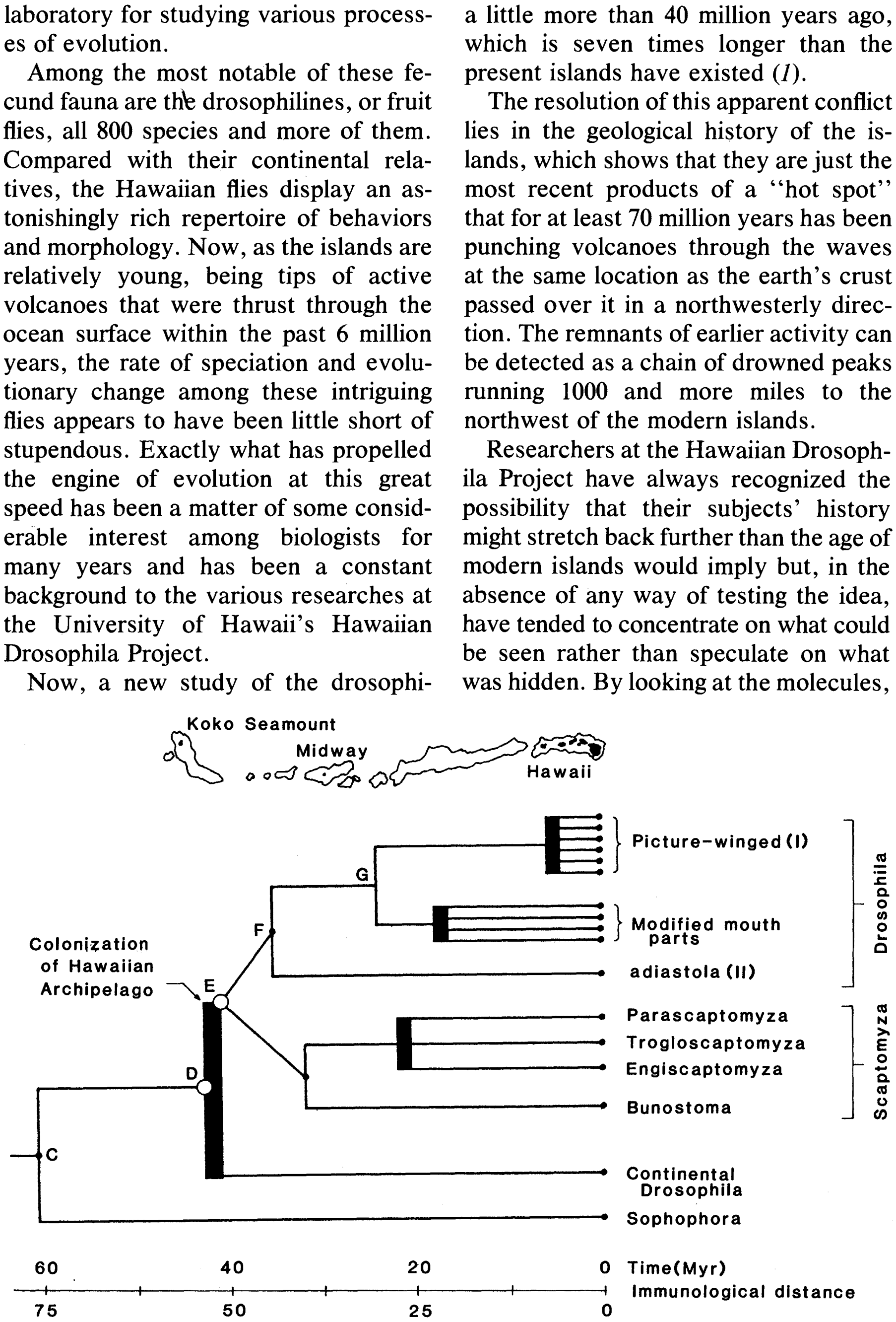
The Hawaiian Islands, strung out as a 400-mile chain running northwest to southeast 2000 miles from any major landmass, have long fascinated biologists. Colonists of myriad organisms—insects, birds, plants—appear to have radiated wonderfully in their splendid Pacific isolation, thus offering a natural laboratory for studying various processes of evolution.

Among the most notable of these fecund fauna are the drosophilines, or fruit flies, all 800 species and more of them. Compared with their continental relatives, the Hawaiian flies display an astonishingly rich repertoire of behaviors and morphology. Now, as the islands are relatively young, being tips of active volcanoes that were thrust through the ocean surface within the past 6 million years, the rate of speciation and evolutionary change among these intriguing flies appears to have been little short of stupendous. Exactly what has propelled the engine of evolution at this great speed has been a matter of some considerable interest among biologists for many years and has been a constant background to the various researches at the University of Hawaii's Hawaiian Drosophila Project.

Now, a new study of the drosophilines, based on their molecules rather than their morphology, has produced something of an answer that at first sight appears to be impossible. According to Stephen Beverley, of Harvard Medical School, and Allan Wilson, of the University of California, Berkeley, the Hawaiian flies started their lives on the islands a little more than 40 million years ago, which is seven times longer than the present islands have existed (*1*).

The resolution of this apparent conflict lies in the geological history of the islands, which shows that they are just the most recent products of a "hot spot" that for at least 70 million years has been punching volcanoes through the waves at the same location as the earth's crust passed over it in a northwesterly direction. The remnants of earlier activity can be detected as a chain of drowned peaks running 1000 and more miles to the northwest of the modern islands.

Researchers at the Hawaiian Drosophila Project have always recognized the possibility that their subjects' history might stretch back further than the age of modern islands would imply but, in the absence of any way of testing the idea, have tended to concentrate on what could be seen rather than speculate on what was hidden. By looking at the molecules, specifically at immunological properties of larval hemolymph protein (LHP), Beverley and Wilson were able to track and apply a time scale to a history that from all other physical points of view is essentially lost or inscrutable.



Molecular tree for Hawaiian drosophilines in relation to time and geography

The exact time of colonization of the islands is uncertain, but is thought to represent one event, followed by divergence into the two major groups.

Beverley and Wilson chose LHP as their target because, as an amino acid storage protein for the pupal stage, it is very widespread in the insect world. It is easily isolated and purified, once one has the larvae to hand, which can sometimes present a problem with species not readily bred in the laboratory.

In an earlier stage of their work the researchers had demonstrated that the protein works well in the immunological distance technique, which depends on pairwise comparisons of specific anti-LHP reactions (*2*). For instance, sera raised against LHP of *Drosophila crucigera* will react strongly with *D. punalua*, with which it is closely related, but only weakly with that of *Scaptomyza elmoi*, which is a distant cousin. Differences in strength of reaction is translated to immunological distances, which, through calibration against biogeographic events, yield species' divergence times.

As clock-like behavior can never simply be assumed in any particular protein, Beverley and Wilson had to check that immunolgical distance accurately reflected amino acid substitutions in the proteins and that mutations were accumulated at a steady rate through time. Both criteria were met, as was a general comparison between the tree constructed using the molecular data and one based on morphology.

The protein tree, including divergence times, reveals the general division of Hawaiian drosophilines into the two major groups, genus *Drosophila* and genus *Scaptomyza*, as shown in the diagram. The origin of the two groups is a little more than 40 million years ago, at which time the islands associated with the Koko Seamount would have been above the waves. The uncertainty in the precise time of origin of these two groups means that one of the great puzzles of Hawaiian drosophilines—to wit, was there one colonization, which subsequently split into the two groups, or two separate colonizations?—cannot be settled definitively from these data. For reasons of parsimony, and evidence of internal anatomy compiled by Lynn Throckmorton of the University of Chicago, Beverley and Wilson prefer the idea of a single colonization.

Exactly which of the several continental groups of drosophilines represents the ancestor of the Hawaiian flies has been a matter of contention for many years, with a number of different candidates being championed on different grounds, including morphology, chromosome structure, and behavior. Beverley and Wilson's immunological distance data, however, show that none of the continental candidates is any closer to the Hawaiian flies than is any other. In other words, the Hawaiian flies and the various extant continental groups all appear to have derived from the same common ancestor.

One of the striking features of the Hawaiian drosophilines, particularly among the picture-winged group, is the tremendous morphological and behavioral variations displayed compared with those on the continent. Courtship behavior among the picture-winged flies can be quite elaborate, including territorial defense by males, or lekking.

The picture-winged group is in fact comprised of two subdivisions, which have been considered to be closely related, with a third group, the modified mouth-parts flies, being somewhat more distant. According to the molecular evidence, however, divisions I and II of the picture-winged group diverged some 35 million years ago, and the modified mouth-parts group is a branch of division I, having split away from it a little more than 20 million years ago. This phylogenetic reconstruction differs from conventional ideas based on morphology and on chromosome banding, and is therefore somewhat controversial.

Another controversial result is the placement of *D. amplilobus* (subgenus *Engiscaptomyza*) with the *Scaptomyza* rather than the *Drosophila*, in which it is traditionally classified. From external morphology, the fly aligns itself closely with *Drosophila*, but its phylogeny lies elsewhere, which is interesting in itself.

Overall, then, the phylogeny based on molecules gives Hawaiian drosophilines rather longer than the conventional 6 million years in which to diversify into 800-plus species. The question of their unusually rapid evolution therefore recedes somewhat. However, the extant members of the picture-winged group, division I, of which there are several hundred species, do appear to have arisen within the past 5 million years, and so the question of rapid evolution does ap-

Reverse Transcriptase in Introns

The great majority of intervening sequences, or introns, which interrupt gene sequences in eukaryotic and some prokaryotic organisms, appear to be incapable of coding for proteins. However, a few introns do encode proteins of their own, which in some cases are involved in precise excision of the intron from the messenger RNA precursor. Now, two European researchers, Francois Michel and Franz Lang, of CNRS, Gif-sur-Yvette, France, and the University of Munich, Germany, respectively, report that four protein-coding sequences from introns they have examined may be able to code for the enzyme reverse transcriptase, which makes DNA copies of RNA templates (*1*). The discovery of these sequences, which match reverse transcriptase sequences in retroviruses, has important implications for ideas about the origin of introns and of retroviruses too.

One viewpoint on the origin of introns is that they have been present as long as genes have existed, and have been lost from some of the simpler organisms, the prokaryotes. An opposing notion is that introns are of relatively recent origin and have simply inserted themselves into genes. This simplistic description tends to obscure the fact that there are several different types of introns—those in transfer RNA, in nuclear messenger RNA, and in organelle messengers—which differ in structural characteristics, apparent modes of splicing from precursor molecules, and possibly in their origin. The introns that Michel and Lang have been studying are from fungal mitochondria, which have two classes—I and II.

The first hint that reverse transcriptase might be found in sequences other than those associated with retroviruses came from Alan Lambowitz and his colleagues at St. Louis University Medical School, when they described the sequence of a mitochondrial plasmid from the fungus *Neurospora crassa* (*2*). They noted similarities in the sequence with those of retroviruses and certain mitochondrial introns. Michel and Lang took this further and compared in detail a series of seven regions of conserved sequences in the open reading frames of four class II introns and showed that they matched very closely with similar regions in the plasmid sequence and the reverse transcriptase–coding region of several representative retroviruses. The "reverse transcriptase" region of the introns represents just one-third of their coding length.

The question is, do these mitochondrial introns actually generate reverse transcriptase? And, if so, what does it do? So far there is only indirect evidence that reverse transcriptase is synthesized in fungal mitochondria and takes part in transposition of the intron. One piece of data is the existence of the *N. crassa* mitochondrial plasmid, which looks like a DNA copy of an intron. Another is the occasional excision of an intron from its host gene, which could be accomplished by an interaction with a complementary DNA copy of the spliced message. In both these cases one can infer that reverse transcriptase has been active in the organelle, though there are alternative explanations.

The putative existence of reverse transcriptase in cellular genes offers a source of this enzyme in the origin of retroviruses, which have been suggested to have arisen through the hijacking of this essential gene by mobile DNA elements. But, it is perhaps just as likely that what is being seen in these mitochondrial sequences are the remnants of sequences left behind by a retrovirus: in other words, the introns represent a sink rather than a source of the enzyme sequences. Nevertheless, the fact that these reverse transcriptase–bearing introns more than likely also carry the means of their own excision encourages Michel and Lang to speculate that they enjoy a semi-autonomous existence in the mitochondria, occasionally moving around the host genome. They are already looking for signs of DNA endonuclease activity in the introns, an enzyme possessed by retroviruses and crucial to their insertion into DNA targets.—ROGER LEWIN

References

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ply to them. And, remember, it is not just a matter of rapid speciation but of rapid morphological modification too.

The Hawaiian Islands are unusual in providing a tremendous variety of habitats, from sandy beaches, through lava fields and rain forest, to mountain peaks. Such a habitat mosaic is conducive to frequent speciation, particularly if overlaid upon this is a constant shifting, a conveyor belt of islands that enforces repeated island hops as old peaks become submerged and new ones in the chain rise above the waves. Beverley and Wilson cite several other factors that might be influential in driving evolution, including genetic systems that are particularly susceptible to changes in gene regulation, complex social behavior, and predation pressure, particularly from the burgeoning species of birds.

If Beverley and Wilson's proposed ancient origin model for the Hawaiian drosophilines is correct, then in all likelihood many of the islands' other speciose fauna will also have colonized earlier than 6 million years ago and island-hopped through the ages. This is directly testable through comparisons of molecular data, which, unfortunately, have not yet been collected to any great extent. There are some DNA/DNA hybridization data on the famous Hawaiian honey creepers, a very diverse group of birds, which indicate a divergence time as old as 20 million years ago (*3*). So this at least would fit with the ancient origin model. Further tests of the hypothesis await further data.—**Roger Lewin**

References

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A Spatially Resolved Surface Spectroscopy

A scanning tunneling microscope not only images atoms on surfaces, it may also yield local surface electronic and vibrational spectra

So far it is more promise than proven, but surface scientists have begun reporting the first experiments hinting that the scanning tunneling microscope (STM) can obtain spectroscopic information on surfaces with a spatial resolution comparable to the size of individual atoms and molecules.

To take one example of the value of spectroscopy combined with the atomic-resolution topographic images the STM can already generate, the spectroscopic capability in principle would allow the unambiguous identification of the location and composition of adsorbed molecules. In this "fingerprinting" example, which has yet to be realized, one could associate a vibrational frequency with a bump in the image and conclude, "yes, that's carbon monoxide," and so on.

Identification of adsorbed molecules by their vibrational spectra is a particularly clear-cut example of the sybiosis between imaging and spectroscopy whose value is undoubted. Identification of the electronic quantum states of surface species is another potential application. While experiments testing this possibility have already been reported, how easy it will be to interpret the results remains an open question because there is little theory available as a guide.

At the very least, even without detailed interpretation, spectroscopic differences between neighboring areas of a surface give a clue as to what is going on. Spectroscopically, for example, a clean nickel surface looks different from an oxidized one, notes Heinrich Rohrer of the IBM Zürich Research Laboratory, where the STM was invented in 1981. "If spectroscopic features associated with a bare surface disappear during scanning, we know we are moving into an oxygen-covered area," says Rohrer.

The STM works by the quantum mechanical phenomenon of tunneling. A metal (usually tungsten) wire with a finely ground tip is mechanically rastered across the surface of the sample. If the tip is close enough, a few angstroms, the wave functions of the electronic quantum states of the atom or atoms at the end of the tip and of those near the surface overlap slightly. The overlap allows electrons to jump across the vacuum gap between the tip and surface when a voltage is applied between them, producing a tunneling current, which decreases exponentially as the gap distance increases.

By keeping the current constant with an electronic feedback circuit, the tip is maintained at a fixed distance above the surface during scanning. In this way, the trajectory of the tip traces out a profile of the surface, including, in an increasingly routine way, the bumps due to individual atoms (*1*).

While the STM is conceptually simple, implementing the idea is not so easy. The relative position of the tip and surface must be controlled to within a fraction of an angstrom. The best instruments can now maintain the separation between tip and surface with a jitter of less than 0.1 angstroms, for example. Piezoelectric materials that expand or contract when a voltage is applied take care of the scanning. But it is also necessary to shield against vibrations from outside the instrument and mechanical resonances induced by motion within the instrument.

As it happens, quite a few researchers have taken the plunge and invested the time and effort to get into the STM game. Just in the United States there are three instruments at IBM's Yorktown Heights Laboratory and others at AT&T Bell Laboratories, the National Bureau of Standards, the Ford Motor Company, the University of Virginia, Stanford University, and the University of California campuses at Santa Barbara and Berkeley. And early in July, IBM sponsored a workshop that attracted about 60 European and American surface scientists to Lech, Austria, where they swapped STM experiences.

The one feature that the STM brings to surface investigations that other techniques (with the exception of high-resolution electron microscopy) lack is the ability to look at small areas of the surface. The best lateral resolution reported so far comes from the Zürich laboratory, where several IBM physicists collaborated to image the surface of pyrolytic graphite with a new "pocket-sized" STM constructed by Christoph Gerber (*2*). The images showed not only that the carbon atoms are arranged in the well-known honeycomb pattern with a diameter of 2.4 angstroms, but also that the six carbon atoms making up a ring are not equivalent. The difference stems from the fact that three of the carbons have neighboring atoms in the layer immediately below, whereas the other three do not.