

Hawaiian *Drosophila* Project

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I. ORIGINS

The Zoology Department of the University of Texas has been deeply involved in the study of *Drosophila* from the early 1920's to the present. During the 1920's Herman Muller, then a member of the faculty, utilized x-rays to create mutations in *D. melanogaster*, for which he was later awarded the Nobel prize; Theophilus Painter (1934) was the first fully to elucidate the bandings in the polytene chromosomes of *melanogaster* and their significance. From 1933 until his death in 1960, J. T. Patterson, known to his friends and students as Doctor Pat, vigorously studied the systematics, biology and evolution of numerous species of the genus *Drosophila*. Although by 1935 *melanogaster* was widely used by the geneticists in all parts of the world as an experimental organism and a few investigators used other species (e.g., *pseudoobscura*, *persimilis*, *simulans*, and *funnebris*), geneticists as a group had no detailed knowledge of or interest in the biology and systematics of numerous other drosophilid species, many of which were undescribed. Likewise the entomology fraternity had also paid scant attention to *Drosophila*. Alfred Sturtevant was a major exception. Since his graduate student days at Columbia under Morgan, he had devoted time and thought to the North American species.

Patterson felt that a serious scientific lacuna existed and he set about in his usual vigorous and determined manner to change the existing situation. He attracted to Texas a group of outstanding colleagues and graduate students, such as Wilson Stone, Marshall Wheeler, James Crow, T. C. Hsu, Robert Wagner, Dean Parker, William Baker, Lynn Throckmorton, William Heed, Sarah Bedicheck Pipkin, Frances Clayton, Gordon Mainland and Marvin Wasserman. This list is incomplete and merely cited to show the quality of the individuals whom Patterson and later Patterson and Stone selected to participate in the studies on *Drosophila*.

In 1945, at the urging of Theodosius Dobzhansky and Ernst Mayr, I began to study the courtship behavior of *Drosophila* species. In 1949-50 I spent a sabbatical year at the University of Texas, which I selected because of the large number of species available in its collection of *Drosophila*. Patterson was retired, but still enthusiastically engaged in research and the great landmark opus EVOLUTION IN THE GENUS DROSOPHILA, published with Stone in 1952, was germinating in his mind that year. He consistently arrived at the laboratory at six in the morning, a practice that he had followed for decades, and by 9:00 a.m. he had accomplished about the equivalent of a full day's achievement for most scientists. He was then ready to talk about *Drosophila*, evolution, the history of zoology or some other zoological subject with one or more of his colleagues. I was many times privileged to listen and talk with him. He knew thoroughly the literature on drosophiloid systematics and biology,

including the publications of Perkins, Grimshaw and others on the Hawaiian *Drosophila*, and he was interested in stimulating and facilitating investigations of the Hawaiian fauna. In fact, a few years previously (1946) his student Gordon Mainland had accepted a position in the Department of Zoology at the University of Hawaii.

Mainland who had received his M.A. in Hawaii returned with the express intent of studying *Drosophila* and immediately proceeded to do so. At that time a rich native drosophiloid fauna still existed in the area of Tantalus and Manoa Falls on Oahu, and he was able to collect numerous species. Like everyone else who had previously attempted to rear the various species under laboratory conditions, he was unable to do so. Eventually he became disillusioned with the Hawaiian *Drosophila* and with his duties as an investigator on the Oriental fruit fly project. By 1949 he had resigned and returned to the mainland.

Patterson still had hopes that in the future methods of rearing the Hawaiian species could be developed, especially since a number of breakthroughs had been achieved during the preceding years in rearing species from other areas that hitherto had been recalcitrant to laboratory conditions.

Wilson Stone and I also developed a close and enduring friendship during that year of 1949-50 and we talked often about the Hawaiian flies. We agreed that within the next several years we should make another attempt to rear the flies in the laboratory. Events dictated that it would be more than a decade before the next attempt was made since Stone became involved with the Atomic Energy Commission activities in the western Pacific and I became a full-time university administrator.

Fortunately, however, D. Elmo Hardy had joined the University of Hawaii Department of Entomology in 1948 as specialist on systematics of fruit flies (Tephritidae). Although his major research interests were in the Tephritidae of the Oriental and Pacific regions and in various families of parasitic and herbivorous flies of the tropics of the world, soon after arrival in Hawaii he began work on a five volume series on the Diptera as part of the Insects of Hawaii series. He was introduced to the Hawaiian drosophiloids by Gordon Mainland. Hardy began to collect the native *Drosophila* species and quickly came to the realization that here indeed was a most remarkable fauna and a unique biological situation which deserved intensive study and investigation. During these years Stone and Wheeler made numerous trips to the Marshall Islands in the Pacific. Often they had "stop overs" in Hawaii and would visit with Hardy. They were impressed with the number of new species Hardy was collecting. Robert Usinger, University of California, Berkeley, also encouraged Hardy on several occasions to seek funding for a project on the evolution of Hawaiian drosophiloids. Although not directly related to the Hawaiian Project, Zimmerman's 1958 paper in *Evolution* (12:557-558) stimulated appreciation of the size and complexity of the Hawaiian drosophiloid fauna. For a number of years Hardy used the story of the amazing Hawaiian fauna as the subject for seminars, talks and discussions in the United States and Europe. In 1962 he visited the University of Texas and discussed the possibility of organizing a research project with Stone. After

talking for ten minutes Stone said "Let's do something about it." So proposals were prepared and submitted to N.I.H. and N.S.F. for a cooperative project between the University of Hawaii and the University of Texas on the evolution and genetics of Hawaiian *Drosophilidae*.

It is clear that Patterson is the intellectual ancestor, so to speak, of the Hawaiian *Drosophila* project; Hardy and Stone are the functional parents. Hardy's visit to Stone was the catalyst that started the project on its way.

Stone telephoned me in the spring of 1963, telling me that the grants requested by Hardy and him had been funded, to begin July 1, 1963, and he said that he expected me to be one of the individuals who would be involved in the research. I replied that as an administrator I had only one free month. In vigorous Texan language, he noted that was about eleven months short of what was needed, especially since the grants were for only one year with the condition that they might be extended if, during the first year, progress was made on the vexing and hitherto intractable problem of rearing the species in the laboratory. Obviously, the granting agencies knew about Mainland's and other investigators' experiences. That evening I decided I did not intend to miss out on this exciting investigative proposal, and I resigned as Chancellor of the Riverside campus of the University of California and returned to faculty status.

II. HISTORY OF THE DROSOPHILA PROJECT

The group that assembled at the University of Hawaii in June of 1963 consisted of the following individuals: Marshall Wheeler, University of Texas; Frances Clayton, University of Arkansas; Lynn Throckmorton, University of Chicago; William Heed, University of Arizona; Hampton Carson, Washington University, St. Louis; Harrison Stalker, Washington University, St. Louis and Herman Spieth, University of California, Riverside plus the principal investigators Elmo Hardy and Wilson Stone.

The latter two had made logistic and material preparations for the research project, which included a Jeep Wagoner, sundry equipment and supplies, and research space in Snyder Hall on the Manoa campus of the University of Hawaii which consisted of a large laboratory room, Room 118, and a preparation area across the hall from the laboratory which included two small rooms, one of which had storage facilities and a sterilizer; the other had facilities for washing glassware and preparing media.

Two undergraduates were employed to provide assistance for making food, washing glassware, and assisting with the field studies. Tom Ohta served as field and laboratory assistant and Linda Davis as food preparator and dishwasher.

The over-riding problem was the development of a method of rearing the flies under laboratory conditions, but we quickly became aware that there existed other unique and refractory problems demanding solution. These problems can be categorized as follows: (1) collection of specimens in the field; (2) transportation of the captured flies to the laboratory and the shipping of living specimens to Texas; (3) maintaining the captured

flies in healthy condition in the laboratory; and (4) identifying the species.

Hardy had had extensive collecting experience and he quickly educated us to the fact that collecting native Hawaiian drosophilids demanded different techniques and procedures from those that we had used on the mainland. First, the flies dwell only in the cool, wet rain forests located at higher elevations and, secondly, the individuals are not attracted to the baits of rotting bananas or similar substances that had been used successfully in most other parts of the world. Hardy himself and other Hawaiian collectors captured their specimens by using nets, typically heavy "beating" nets which they swung vigorously through vegetation close to the ground. This vegetation typically consisted of bracken ferns and some low shrubs. The investigators also kept a sharp lookout for individual flies sitting on the vegetation, especially on broad-leafed trees and shrubs. The beating method often injured the flies; such flies might serve well as pinned specimens but not for laboratory studies. Since the flies would not come to any of the "standard" baiting materials, we perforce at that point in time had to use nets. First, of course, we had to reach a site where the flies were living. Until one has personally engaged in collecting Hawaiian flies, it is difficult to appreciate how expensive in time, energy, and money such collecting is.

The higher elevations of the volcanic cones are highly dissected by steep, often sheer walled valleys as well as by lava flows. The terrain is rough, the footing treacherous, and the vegetation in many places so dense as to be almost impenetrable. At least 98% of the Hawaiian human population lives below the 1,000 foot level. In a few exceptional places such as Kokee on Kauai, Olinda and Kula on Maui, and Volcano on Hawaii, humans dwell permanently at elevations that reach 4,000 feet, but these areas have been cleared of much of the native forests. Essentially no one dwells in the rain forests and few people enter the forests except the military, pig hunters, foresters and lumbermen, water officials and a few scientists. Access routes to the forests are under the control of private owners, the State foresters and water authorities, or (on Oahu) the military. Therefore permission must be secured from one to three different agencies to reach a collecting area.

The access routes themselves usually require a Jeep or comparable vehicle because the roads are narrow, steep, rough, and unpaved. Even they often end some distance from the actual collection site. Trails leading from the road head usually exist only because of the needs of the foresters and especially the water service personnel. They are steep, rough, and narrow. Half a day is frequently needed just to traverse the distance from the coast to the collecting site. Surprisingly, Oahu (where the University is located) was and still is the most difficult of the islands upon which to collect.

When we went to one of the other islands, we had to fly from Honolulu, land, pick up our equipment, food, etc., and then head for the hills. If we needed the Jeep, we would have to ship it by inter-island barge a week before the date of the field trip.

Fortunately, there exist three exceptional sites that can be reached by paved roads and which are also excellent collecting sites, with living

accommodations nearby. These are Kokee State Park on Kauai, Volcanoes National Park at Volcano, Hawaii, and the Tantalus area at the head of Manoa Valley on Oahu just above Honolulu. During the early part of the century, the Tantalus area was the major spot for collecting *Drosophila* and it was here that Mainland did most of his work. Unfortunately, by 1963 the majority of the native *Drosophila* species had been exterminated, apparently by the intrusion of exotic vegetation which decimated the native flora, and especially by ants which also had unfortunately been introduced. Wherever ants, a group totally lacking in the native fauna, become established, the *Drosophila* are essentially eliminated. Nevertheless, we spent considerable time during 1963 in the Tantalus area and visited a few other spots on Oahu such as Mt. Kaala and the Castle Trail. Our most productive collecting was at Volcano, on the island of Hawaii, working in the National Park, particularly in Kipuka Ki and Kipuka Puaula (Bird Park). Kipuka is the Hawaii word for "opening" and is used to designate an area that has been surrounded but not covered by one or more recent lava flows. A kipuka is an island of limited size on which older forests grow on deeper and richer soils than that of the surrounding terrain. In a real sense kipukas are natural experimental plots. There are many kipukas, especially on the island of Hawaii. Varying in size and age they have been of considerable value to a number of investigators (see below).

When we captured the flies we followed the standard technique of placing them in small vials with a layer of food media on the bottom of the vial. We found that the flies, unlike mainland species, often became stuck to walls or the food. It also became apparent that they could not tolerate the temperatures under which mainland flies thrive. Surprisingly, the Hawaiian species demand that the ambient temperature be below 70° F. and preferably 63°-65°F. For shipping we could use insulated boxes with "cold cans," i.e., artificial ice. This tended, however, to cause condensation in the vials in which the specimens were stored, and the "sticking" problem became accentuated.

It was clear that the laboratory would have to be air conditioned, and after several months this was accomplished by installing large window units.

Stone returned to Texas by mid-July and most of us returned to our home institutions before the fall semester started. We had learned something about our problems but otherwise our achievements were modest. Heed had found the larvae of one species breeding in the leaves of *Cheirodendron* at Paliku, Haleakala, East Maui. This was the compass sign to the eventual solution of the larval substrate problem that Heed so successfully pioneered and solved. Throckmorton also had a rewarding summer. For studying the anatomy, especially the internal structure of the reproductive system, he needed freshly captured specimens but he did not have to maintain them alive longer than the period from capture in the field to dissection in the laboratory.

Wheeler and Clayton, both on sabbatical leave, remained in Hawaii for 1963-64. Clayton was interested in the cytology of the flies and Wheeler in taxonomy and biology. Ohta decided to leave the project and Miss Davis went on to graduate school on the mainland. Thus, new undergraduate

assistants were appointed. Throughout the life of the project undergraduates have served an important and valuable role in the conduct of the research; approximately 135 of them have participated to date (1977). A large percentage of them have eventually received advanced degrees in zoology, genetics, botany, medicine, dentistry, veterinary medicine and the paramedical field. One of these undergraduates, Kenneth Kaneshiro, then a sophomore, replaced Ohta in October, 1963. Today he is Dr. Kaneshiro and a senior investigator in the *Drosophila* project. Ken is a superb field man, a gifted systematist, and an insightful experimentalist. I am sure that other senior investigators in the project will agree that Kaneshiro's overall intellectual grasp and knowledge of Hawaiian *Drosophila* are more comprehensive than that of anyone else who has participated.

Wheeler and Clayton concentrated during the fall and winter of 1963 on the problem of rearing the flies. They tested a large number of recipes, many more or less standard ones that at one time or another had been used to rear *Drosophila* from other parts of the world. They also prepared and tried a number that could only be termed exotic. None worked, although they did manage to induce occasional females of a few species to oviposit and were able to keep a stock of *D. mimica* from Bird Park staggering along.

During the period when Wheeler was in the "Valley of Despair," his young daughter Sandy refused to eat the prepared dry cereal that had recently been purchased. Wheeler decided to take the box of Special K to the laboratory and dropped pieces of the cereal into the *Drosophila* food vials. Freshly captured *D. mimica* oviposited readily in the pieces of Special K. Wheeler and Clayton then began to experiment with various cereals and as a result, the Wheeler-Clayton food was developed (DIS 40: 98). The field captured adults of all Hawaiian flies, when placed on Wheeler-Clayton, remain healthy, can be kept alive for considerable periods of time, and a considerable number of species breed well on it. It is today the standard media for the Hawaiian flies (but see below for modification of Wheeler-Clayton, additions, and failures).

Solution of one problem often leads directly to another one and this is what occurred with Wheeler-Clayton larval food. The mature larvae refused to pupate in the rearing vials; rather, they crawled through the cotton plugs of the food vials, wandered around and desiccated. Wheeler and Clayton tried a series of solutions, some of which could only be described as desperation measures. Wheeler remembered, however, that Kenneth Frick had successfully induced larvae of Agromyzidae to pupate by placing the larvae on a layer of sand. From this clue they evolved the "sand jar technique," i.e., they placed two to three inches of moist, sterile sand on the bottom of large glass jars. Then they removed the cotton plugs from those food vials which contained almost mature larvae and dropped the small vials on the surface of the sand in a large jar. The jar was then closed with a tightly fitted piece of cloth. Success. The larvae left the food vials, crawled around on the sand or wall of the big jar, some species for several days, but finally they bored into the sand and pupated in it.

When the adult emerges, the teneral fly wriggles up to the surface, then

expands its wings and matures. Today we know the flies follow the same procedure in their natural forest soils. To everyone's surprise, the emerging adult of one species grasps a particle of sand in its mouth as it emerges from the pupal case and retains it until the fly reaches the surface—after which it spits out the grain of sand.

In the summer of 1964 we were more adept at collecting, although we still depended on netting the flies in the field. A major difficulty was that only Hardy could identify many of the species. Hardy's 1965 massive and basic publication on the systematics of the Hawaiian *Drosophilidae* (Vol. 12, *Insects of Hawaii*: 814pp.) was available in galley proof but the extremely close morphological similarity of many of the Hawaiian species, especially the females of the species, created problems that only Hardy could solve.

Throckmorton effectively pursued his anatomical studies and by the end of the summer was able to identify and delimit the major species groups. He designated the various groups by name derived mainly from unique structures, especially male secondary sexual structures which were shared by all species of each species group, e.g., spoon tarsi, split tarsi, bristle tarsi, picture-winged, light-tipped scutellar. A few of the species groups were so distinctly different that Hardy and Grimshaw had placed them in distinct genera, e.g., *Antopocerus* and *Idiomya*. Throckmorton further concluded that all the numerous Hawaiian species had descended from a single ancestral population and, in fact, that the genus *Scaptomyza*, which has more species in Hawaii than elsewhere on the globe, probably arose from the same ancestral immigrant.

The Hawaiian *Drosophilidae* are intriguing to evolutionists and to geneticists not only because of the large numbers of species found on such small land masses, but also because of the amazing anatomical differences and especially the large size of the individuals of many of the species. Throckmorton's data confirmed Hardy's previous studies that the largest species all belong to *Antopocerus*, *Idiomya*, and the picture-winged flies. It also became apparent that the picture-winged species were numerous on all the islands and that many of them could be successfully reared on Wheeler-Clayton media.

Harua Tokada of Kushiro Women's College, Hokkaido, Japan joined the project for the summer of 1964. He studied the male genitalia of 55 species of drosophiloids, utilizing both drosophilid and scaptomyzid representatives. His studies complemented Throckmorton's efforts and helped greatly to elucidate the relationships between the various species groups.

Heed systematically began to search the native vegetation for the ovipositional sites, i.e., larval substrates. The native plants in the forests do not produce large fleshy fruits that can serve as larval substrates. The two most common trees in the forests, *Metrosideros collina polymorpha* and *Acacia koa*, occasionally develop slime fluxes on their trunks or limbs (at Kokee on Kauai *D. picticornis*, a picture-winged, was found in *Metrosideros* fluxes), but basically both of these trees were eliminated as prime larval substrates. Less numerous but rather uniformly distributed in the forest are the broad-leaved evergreen trees *Cheirodendron* and it was in their rotting leaves that Heed found numerous species of drosophiloid larvae.

Subsequently the related genera *Tetraplasandra* and *Reynoldsia* were found also to be used as larval substrates. Heed found several other substrates and set the pattern for the further discovery of substrates.

Various types of fruits, vegetables and other substrates were tested as baits to attract the flies in the field, but all were indifferently effective. Carson particularly concentrated on this area and it was learned that in another characteristic the Hawaiians differ from their relatives in most other parts of the world. Thus, on the mainland we place baits in the bottom of buckets, cans, or other containers, and collect the flies by kicking or otherwise disturbing the container, thus stimulating the flies to fly upward where they can be caught in an insect net. When the Hawaiian flies are disturbed, they fly *downward* and become enmeshed in the baits. Carson therefore began to smear or attach the baits to boards or pieces of heavy cardboard, attaching these in a vertical position on trees or other objects. He also found that the larvae of the picture-winged flies, especially the *grimshawi* stocks from Molokai and Maui which breed well in the laboratory, possess large and excellent salivary chromosomes. He therefore took the picture-winged species group as his province for investigation, concentrating on the chromosomal polymorphisms.

Stalker did not return to Hawaii in 1964. Clayton, however, was now able effectively to study the cytology of the various species that could be reared in the laboratory. Both she and Carson were further helped by the fact that mature females of a number of species, especially some of the picture-winged flies, when captured in the field and brought into the laboratory, would oviposit. The eggs would hatch and the larvae develop on Wheeler-Clayton food. These could be used for investigations but the F₁ larvae would either not mature or if they did produce F₁ adults these would not oviposit although they might live for a considerable period of time; thus the stock would die off.

In the Fall of 1964 I was on sabbatical leave, so I remained in Hawaii after other investigators had departed at the end of the summer. During the summer I had made observations on the courtships of several species and was frustrated because although the males might court vigorously, the females were almost all uniformly recalcitrant and refused to copulate. This occurred with both field captured and laboratory reared virgin females. Some species simply refused to engage in sexual activity in the laboratory. I also observed that the flies, especially the males of most species, showed intense antagonism toward each other, engaging in behavior that could only be described as fighting. This occurred also in the rearing vials, and the large picture-winged flies often broke each others' wings.

Since I knew that mainland flies engage in courtship and copulations on the feeding sites in the field, I decided to spend most of the fall in searching for feeding sites as well as collecting flies, etc. I chose three places: Kokee on Kauai, Waikamoi on East Maui, and Bird Park at Volcano, Hawaii, each known to have populations of several species.

At Kokee I was able to collect flies but could find no feeding sites. Therefore, ground-up *Cheirodendron* leaves and other baits were used and

observed for long periods of time. To my surprise the flies approached the baits in a quite different fashion than expected. Mainland species fly to the baits, often buzzing and circling before landing. In comparison, the Hawaiian species approach cautiously and slowly; they make quick, short darting flights, land, sit immobile for a period of time, then walk slowly forward and finally after many minutes reach the bait. Once there they feed quietly, engaging in no sexual or antagonistic activities. After feeding for a time, they depart by a quick downward flight and disappear.

At Waikamoi on Maui I searched for food sites and came across a spot where a number of tree ferns had been cut down a few days previously, and the stumps were covered with sap. A number of *Clermontia* shrubs had also been broken and cut with the result that the milky sap was exuding. Large numbers of flies of several species were ravenously feeding on these saps. Again, it was the same behavior as at Kokee. The quiet, cryptic behavior of the flies was amazing. I observed no courtships or antagonistic behavior by the flies when they were feeding. Neither did I find any courtship sites.

At Kipuka Ki, Volcano, a large limb bearing fungus had fallen and here the flies were also quiet, but as soon as they moved off the fungus food site they became antagonistic. I observed males sitting on the upper surface of nearby leaves. Whenever another individual alighted, the male approached and courted. In another part of Kipuka Ki a large *Polyporus* fungus was found and again the pattern of behavior was similar to that found on the other fungi. Thus, the flies separate feeding and courtship; i.e., they engage in lek type courtships.

On the lek the males are aggressive, defending it vigorously if another male enters it. The females are extremely choosy in the selection of a male. Even when they are sexually ready and enter a lek, they will often allow a male to court for long periods and then depart without copulating.

We still had trouble transporting flies to the laboratory and especially in shipping them to the University of Texas laboratory. That fall I developed the sugar vial (DIS 41: 146-147) and the vexing problem was solved. Now flies could be transported safely and effectively to all parts of the world.

At the end of April in 1965, John Murphy came from Texas to serve as the operational manager of the project. Intelligent, tactful, and with a vivacious personality, he quickly became the individual to whom everyone turned for logistic planning and execution, both in the laboratory and in the field. The space we were occupying in Snyder Hall was needed for other functions but there existed on the campus a building known as the Auxiliary Services Building. This structure was built to serve as the space for storage and initial preparation of food for the various cafeterias on the campus. It can be described as being "ahead of its need" and we were able to obtain two large air conditioned rooms equipped with stainless steel tables and sinks. Off each of these rooms are large walk-in freezer rooms and other spaces. A third room was converted in 1966 into a microbiology-biochemistry laboratory. In addition, three small offices and a storage room were assigned to the project. It truly became an ideal location for our investigational purposes.

Malcolm Brown, a graduate student specializing in microbiology in the

Botany Department at Texas, arrived in Hawaii in June of 1965 and began an investigation of the yeasts and other microorganisms found associated with the native vegetation and especially those found on or in the decomposing substrates in which the *Drosophila* larvae were living or upon which the adults fed, especially *Cheirodendron* and *Clermontia*. He isolated a number of the favored yeasts and collectors then began to hang petri dishes which had been inoculated with these yeasts in the field as baits. This improved the baiting techniques somewhat.

The Hawaiian species are cryptic, in both their coloration and behavior. The adults often sit motionless on the vegetation for prolonged periods, especially in the areas surrounding a natural food source. If disturbed they typically fly or dive downwards. Kaneshiro and Jack Fujii exploited this behavior of the flies. They searched the vegetation, moving cautiously and deliberately. Finding a specimen on the underside of a limb or leaf or on the trunk of a tree, they slowly thrust the open end of an 8 dram glass vial over the fly. Invariably the specimen then dove to the bottom of the vial. This technique became known as the "pick off" method. Later the use of glass tube aspirators was introduced and today these two techniques are the standard methods for collecting those species that are attracted to baits as well as those individuals sitting on the vegetation. For those species for which we have as yet no effective baits, sweeping with nets is still mandatory.

Two persistent problems continued to exist in the rearing of the flies: (1) The food in the large shell vials known as Texas vials became moldy and this smothered the eggs which had been deposited into them and also debilitated the adults; (2) the adults tended to "stick" to the food or the walls of the vials. Our normal practice was to insert into the food a small amount of loosely folded Kleenex tissue, with the major part of the paper extending upward above the food; i.e., this portion served as material for the flies to sit on, thus preventing them from becoming "stuck."

Kaneshiro developed the method of folding a small piece of Kleenex into a rectangular pad of about 1 x 2 inches, moistening this with a sterilized solution of yeast, sugar water, and propionic acid. This pad was then pressed against the inside wall of a vial and almost in contact with the food at the bottom. The food vial was then laid on its side with the pad on the "bottom." Thus, the adults could sit on the pad and feed when they desired on the vertically oriented food.

At Texas, Stone with the aid of Kathleen Resch was engaged in building up stocks of the Hawaiian species. Kathleen is an energetic, intelligent and imaginative lady, unquestionably the best "rearer" of Hawaiian *Drosophila* alive today. Suffice it to say that if she cannot keep a species alive and healthy in the laboratory, no one can. Her skill has been invaluable in making it possible for various investigators, especially those at Texas, to study and publish findings on species and species groups that otherwise would not have been available. Kathleen spent the period of February-July, 1966, in the laboratory at Honolulu. She had earlier determined that by immersing fresh *Cheirodendron* leaves in distilled water soluble fungistatic substances escaped from the leaves. This became known as *Cheirodendron* "tea." She also suggested that dampened Tomac tissues which are

aseptic be used for making the pads rather than Kleenex.

Kaneshiro then methodically made teas from the leaves and stems of all of the various native plants that we knew were used as ovipositional substrates. Not surprisingly, at least in retrospect, he found that all of these contained fungistatic substances and also that the teas provided ovipositional stimulus to the females. *Cheirodendron* tea proved to be the least effective and the many species that use *Cheirodendron* leaves as their ovipositional sites still remain recalcitrant to oviposition in the laboratory. *Clermontia* tea is the most effective and broad spectrum for mold control and ovipositional stimulation. Another advantage of the pads is that the females prefer to oviposit in the paper. When the larvae emerge they migrate to the food.

Heed arrived in Hawaii in January, 1966, for a sabbatical and immediately began an intensive search for larval substrates. In February he found that the rotting bark of the lobelioid *Clermontia* was the substrate used by a number of species. This led to finding other species utilizing the related lobelioids *Cyanea* and *Lobelia*. He had earlier discovered that *Cheirodendron* leaves were used by a number of species, and in June he found *Cheirodendron* bark also serving as a substrate, which indicated that a large variety of plants might be used. Eventually he reared 166 species of drosophilids and scaptomyzids from 34 families of plants. Most of the species were monophagic but a few were extreme polyphags.

Forbes Robertson of Edinburgh, who had had considerable experience in the area of *Drosophila* nutrition, joined the group from May to late August of 1966. He chose to investigate *D. waddingtoni*, then known as *D. disticha*, which uses decaying *Cheirodendron* leaves as its larval substrate and whose eggs, larvae and adults can be readily collected in numbers at Waikamoi, East Maui. Robertson made the significant discovery that while the adults use yeasts, the larvae of *waddingtoni* feed exclusively on bacteria. He was, however, unable to rear stocks of the species in the laboratory. Even today, twelve years later, we still cannot maintain stocks of the varied and numerous species which use *Cheirodendron* as a larval substrate. During the late afternoon of the day that the Robertsons were to depart for Scotland, the laboratory personnel had a picnic for them at Hanauma Bay, and I took the opportunity to talk with him about his investigations on *waddingtoni* and other Hawaiian species. He ended the discussion by saying: "It will be really pleasant to get back to Edinburgh and work with normal *Drosophila* for these Hawaiian species literally make one psychotic."

When Malcolm Brown returned to continue his studies upon the yeasts, he isolated a species from rotting *Clermontia*. Kaneshiro inoculated Gerber's baby banana with this yeast and then used the fermenting banana to smear on the trunks and under surfaces of limbs of trees and shrubs. Such bait proved moderately attractive to a considerable number of species, especially the picture-winged flies. It was easy to use in the field and the method of application allowed the pick-off technique to be employed. This then became the standard baiting and collecting procedure. A number of species, however, especially those that oviposit in *Cheirodendron* leaves, only occasionally come to this bait.

Joseph Grossfield who had just completed his doctorate at Texas, came to Hawaii at the same time as Kathleen Resch. He investigated the field biology of the flies with emphasis on their responses to different light intensities. In doing so he made the valuable discovery that the Hawaiian species are light dependent; i.e., they do not mate in darkness.

The period from 1967 to 1970 was one of great achievements by the various investigators. Carson pursued his elegant studies on the polytene chromosomes of the picture-winged species. Clayton methodically and effectively studied the cytology of many species, concentrating especially on the picture-winged flies. Heed pursued the difficult and often frustrating investigation of the ovipositional substrates and also began his studies on the populations of various kipukas on the big island of Hawaii.

Because the *Cheirodendron* species, i.e., those whose larvae developed on some part of the trees, were so resistant to laboratory rearing it was suggested that perhaps there was some peculiar characteristic in the biochemistry of *Cheirodendron* that was responsible for their behavior. Heed had earlier solved a somewhat similar problem with *D. pachea* which uses the *Senita* cactus. He therefore joined with Henry Kircher, a biochemist at the University of Arizona, in an intensive analysis of *Cheirodendron*. Unfortunately, they did not solve this particular problem but they did learn a great deal about the biochemistry of *Cheirodendron*.

In 1967 Mike Kambysellis, who had just received his doctorate at Texas, arrived in Hawaii. He and Heed soon joined together to investigate what can be broadly described as the reproductive strategies of the species of various species groups. A senior investigator of the project once remarked to me that Heed is probably the one member of the group who has not received adequate recognition for his contributions to the project, and there is substance to this evaluation. All of the problems to which Heed devoted his efforts were complex, elusive, time consuming, and often frustrating—but in every instance he achieved significant and basic information which he analyzed with skill and clarity.

At Texas Mrs. Yang and Wheeler investigated the problem of hybridization, using numerous species of the picture-wingeds. Their valuable studies complemented the work of Carson and gave us an understanding of the relationship of the various species, as well as helping to further delimit the parameter of the species group.

Hardy and Kaneshiro described numerous new species that continued to appear in the collections that were constantly being made. They also determined the geographical ranges, identified sibling species, and gave insight into the speciation process, especially within the picture-winged flies.

Kaneshiro completed his undergraduate studies in 1966 and became a graduate student and functionally a senior investigator as well as the prime field collector and skilled rearer of species in the Honolulu laboratory. His studies on the male genitalia enabled him to delineate clearly the subgroups within the picture-winged flies. This investigation plus data from Carson's studies showed that the species of the genus *Idiomyia* really constitute a subgroup of the picture-winged species group. *Idiomyia* has now been synonymized with *Drosophila*. Kaneshiro also studied the

anomalous species group known as the crassifemur group, showing that it constitutes an intermediate unit between the genus *Scaptomyza* and the genus *Drosophila*, but that it is more closely related to *Drosophila* than to *Scaptomyza*. He then placed the species of the group into the new subgenus *Engiscaptomyza*.

Although Stalker did not return to Hawaii after 1963, he did use the salivary chromosomes to elucidate the relationship of the picture-winged flies to the mainland robusta species group, thus giving a clue to the relationship of the Hawaii flies to their ancestors.

A number of other investigators also were connected with the project during these years for variable periods of time, but I do not list them. Their names are recorded in the "Evolutionary Biology of the Hawaiian *Drosophilidae*" (1970, in *Essays in Evolution and Genetics in Honor of Theodosius Dobzhansky*, ed. M. K. Hecht, W. E. Steere, pp. 437-543, N.Y.: Appleton-Century-Crofts).

Two young investigators broke "new" ground: Carmen Kanapi and Susan Rockwood, both graduate students at the University of Texas, were the first to use allozyme procedures on the Hawaiian species.

My own studies on the mating behaviors of the flies proceeded rather slowly during this period for two reasons: (1) as chairman of the Department of Zoology on the Davis campus, I was deeply involved, especially during each academic year, in rejuvenating the department, and (2) the recalcitrance of the flies in the laboratory was intense. I did observe that freshly caught flies from the field were vigorous and active but that after they had been confined for a week or so they became lethargic. The same pertained with laboratory reared, virginal flies by the time they became sexually mature.

I finally decided that Wheeler-Clayton food as then prepared was inappropriate, at least for courtship studies. Therefore I had the brewer's yeast omitted from the preparation of the food. The change was startlingly beneficial. Kaneshiro quickly followed suit and the rearing of the stocks in the laboratory immediately improved. Today it is standard in the laboratory in Hawaii to use yeastless Wheeler-Clayton food. Significantly this is the only modification that has improved the original formula that Wheeler and Clayton concocted in the winter of 1963. We all owe them a debt of gratitude plus, of course, thanks to Wheeler's daughter Sandy who rebelled at eating Special K. Yeastless Wheeler-Clayton food is excellent for maintaining adults and many, but not all, species will oviposit in the Tomac pads that have been moistened with *Clermontia* "tea," commonly also called "juice." The young larvae migrate to the W-C food but it is a rather Spartan larval substrate. Resch therefore formulated a cornmeal food that is much richer and the standard procedure today is to allow the young larvae first to thoroughly "work over" the W-C food. Then a generous amount of cornmeal food is added to the vials. This technique allows a large number of larvae to be reared to maturity in a single vial. The resultant adults from such larvae are healthy and robust individuals. The rearing techniques for the Hawaiian flies are thus more complicated and time consuming than those used for *melanogaster* and other mainland species. Further, the life cycle is long; typically each full generation, i.e.,

from oviposition of the egg to the sexually mature adult, spans a period of at least 40-60 days.

Although the years from 1966-70 were years of accomplishment, there was also tragedy. Wilson Stone died in 1968. His slipping away deprived us of his wisdom, understanding support, and advice. For myself it was not only the loss of a scientific colleague of great stature but also the loss of one of my closest personal friends. No one is indispensable but Wilson Stone certainly approached being that not only for the *Drosophila* project but also for his department at the University of Texas.

John Murphy resigned in 1970 when he accepted a responsible position with the Texas Department of Health Resources. The day to day management of the laboratory then fell upon Kaneshiro, a position which he still occupies. Busy as his days had been, they became even busier and his responsibilities more demanding.

At about the same time that Murphy departed, Steven Montgomery (then an undergraduate) began to work with Heed and others in investigating the ovipositional substrates of the picture-winged flies. This species group consists of over a hundred species and although Heed had found the substrates of a goodly number of the species, there were many he had not discovered. Montgomery is a superb field investigator and he has made invaluable additions to Heed's earlier studies.

In 1970 Carson left Washington University and joined the Genetics faculty at the University of Hawaii, a move which vastly improved his effectiveness in his research for now he not only was constantly in Hawaii but also he was able to have his graduate students and postdoctoral fellows cooperate more fully in the investigation of the Hawaiian species. He continued his studies on the salivary chromosomes and expanded into allozyme investigations and intensively into the problem of the dynamics of speciation, concentrating on closely related species of the picture-wings. In these speciation studies he and Kaneshiro have cooperated closely and effectively.

Heed has turned his attention primarily to the study and identification of the wild yeasts that the larvae use in their substrates as well as to the competition between larvae in the substrates, such as a single decomposing leaf of *Cheirodendron*. These studies of Heed and his graduate students have involved both desert species of Southwestern North America and the Hawaiian species. During a 1972-73 sabbatical at Davis he, in cooperation with Herman Phaff and Martin Miller, investigated various yeasts. He and Francisco Ayala also developed a technique which enabled them to begin to effectively quantify the biology of leaf breeding Hawaiian species.

At Texas Richard Richardson, Wheeler and Yoon, assisted by Kathleen Resch, have studied the salivary chromosomes and the evolution of the crassifemur, antopocerus and part of the modified mouthparts species groups. All of these are exceedingly difficult to handle in the laboratory, but they have been able to elucidate the relationships and evolutionary histories of the crassifemur and antopocerus species groups and also to determine the relationships of the modified mouthparts to other species groups. Their findings have filled large lacunae in our ignorance.

Hardy and Kaneshiro continue their basic studies on the systematics of various species groups. By now large collections have been accumulated and Hardy has revised the *Antopocerus* species group. They are in the process of revising several other groups, basic information which is mandatory for further investigation into the evolutionary dynamics, biology and ecology of the various species groups.

Kipuka Puaulu, known as Bird Park, because of the considerable number of native birds that dwell therein, has been an important research area for the *Drosophila* Project as well as other scientific investigations. It is approximately 56 acres in extent. Until it was incorporated into Volcanoes National Park during the second decade of this century, it was used as a fattening pen for cattle. It is still recovering from the evil effect of such usage and is a prime example of the necessity of the maintenance of preserves which cannot be decimated by the short-sighted activities of human society.

Richardson, aided by his students and associates, has intensively investigated a number of problems in Bird Park and nearby Kipuka Ki which receives less precipitation than does Bird Park.

In 1964 Heed and I observed *Drosophila engyochracea*, a large picture-winged species which has its geographical range limited to the two kipukas and the immediately surrounding area. During daylight hours the flies hide away in the moss found on the trunks of trees. We found that during darkness the flies ascend into the foliage and sit on the undersides of leaves. Spencer Johnston investigated this behavior and found that the flies move to considerable heights in the forests and scatter through the forest—indicating how movements in the population occur.

Richardson then investigated in detail the movements of *D. mimica*, a modified mouthparts species. The adults typically hide in the litter on the forest floors but under appropriate conditions do move; he was able to elucidate these movements and the environmental factors bearing upon such activities. He and his students have also directed their efforts towards understanding the dynamics and possible sympatric nature of speciation that may have occurred. They intensively studied three species that dwell in Bird Park and Kipuka Ki. Their conclusions have attracted attention, including considerable skepticism, but above all they have stimulated others of us to rethink and re-evaluate our ideas as to how speciation may have occurred in the exceedingly rich and complex Hawaiian drosophiloid fauna.

Kambysellis continues his excellent and unique studies on the reproductive strategies of the flies, currently concentrating on allozyme investigations of the eggs of numerous species.

Clayton, despite a long period of debilitating illness, continues to provide new and important cytological data.

I was able to study with the cooperation of Heed, who spent a sabbatical year in Davis in 1972-3, two mainland species, *D. pinicola* and *D. flavopinicola*, which are restricted to the Pacific coastal area. We concluded that they are, on the basis of morphology, physiology and behavior, more closely related to the Hawaiian species than are species of the robusta species group. Since these species oviposit on mushrooms and the adults

feed on mushrooms, I concluded that perhaps rotting domestic mushrooms might serve as a bait to attract the species of the Hawaiian light-tipped scutellar group, often called the fungus feeders. We knew that one of the species, *fungiperda*, was attracted to large *Polyporus* fungi and used such fungi for a larval substrate.

I therefore went back to Hawaii and, although I was myself somewhat dubious about the outcome, my colleagues were downright skeptical about the use of mushrooms as bait. A trial run on Kauai, however, even under adverse conditions, was highly successful. Not only do the fungus feeders come in large numbers to the bait, but also all of the antopocerus, the crassifemur, and the majority of the picture-winged species are attracted in numbers. In typical fashion Kaneshiro quickly improved my original technique of using mushrooms impaled on twigs, i.e., he soaked thin cellulose sponges in "juice" of the rotting mushrooms and then attached them to tree trunks, etc.

The mushroom bait gives us a reliable method of capturing large numbers of individuals of at least 200 species. Unfortunately, mushrooms attract very few of the *Cheirodendron* leaf breeders nor most species of the modified mouthparts and ciliated tarsi species group. This last group is probably the most primitive species group of the Hawaiian fauna and we essentially know nothing about its biology, behavior or evolution at this point in time. The mushroom technique enabled Hardy, Kaneshiro, Ayala and myself to join together in a study on the systematics, allozymes and behavior of the crassifemur, fungus-feeder and picture-winged species groups—a project now under way and yielding considerable new data.

Kaneshiro continues to shoulder the main responsibility for the day to day operation of the laboratory, the organization and logistics of field collecting, and the maintenance of relationship with the various organizations whose help and permission we constantly need—doing all of this in addition to pursuing his own extensive research program. Fortunately, the project acquired in 1976 the services of Mrs. Joyce Karihara. Earlier as an undergraduate Joyce, then Miss Sato, had served as a student assistant. She proved to be competent, resourceful, and responsible. Upon graduation she had become a public school teacher, but after being assaulted and badgered by students, she quit teaching. The Project is fortunate to have her back with it because the student assistants, although quite capable and responsible, work only a few hours a week and each deals with only a limited number of species. Joyce, however, provides continuum and oversight for the laboratory and is invaluable to Kaneshiro and to all the other senior investigators.

A large number of investigators from various parts of the world have participated on the project during the period from 1970 to the fall of 1977, some for short periods, others for prolonged stretches. Their individual research efforts in the main complement and extend the areas of study of one or more of the senior investigators, especially those of Carson and Kaneshiro. The contributions these individuals have made are not discussed at this time but their names are added in alphabetical order as an addendum.

In no sense is this a complete history of the Hawaiian *Drosophila* project. What I have tried to do is (1) to present the antecedents that led to the project's genesis, (2) to show how two intelligent and thoughtful scholars, Hardy and Stone, assembled a group of investigators in order to solve a seemingly intractable problem, (3) how the answers to at least some of the major problems were developed, and (4) the role that serendipity plus that elusive ingredient called insight contribute to the solutions.

ADDENDUM: ASSOCIATE INVESTIGATORS*

| <i>Investigator</i> | <i>Academic Affiliation (1977)</i> |
|---------------------------------------|--|
| Ahearn, Jayne K. (Carson) | University of Hawaii |
| Amnest, Joseph L. (Carson) | University of Michigan |
| Ayala, Francisco | University of California, Davis |
| Baimai, Visut (Carson) | Mahicol University, Thailand |
| Dobzhansky, Theodosius | Deceased, 1976 |
| Fontevilla, Antonio (Carson) | University of Santiago, Spain |
| Jeffery, Duane E. (Carson) | Brigham Young University |
| Johnson, Walter E. (Carson) | Case Western University |
| Johnston, Spencer (Richardson) | University of Texas |
| Kambysellis, Elysee Craddock (Carson) | New York University |
| Loeblich, Karen (Spieth) | San Diego State University |
| Mangan, Robert (Heed) | University of Arizona |
| Newman, Lester (Hardy) | Portland State University |
| Ohta, Alan T. (Carson) | Department of Primary Industries, Brisbane, Australia |
| Raikow, Rody (Carson) | University of Pittsburgh |
| Ringo, John M. (Spieth) | University of Maine |
| Sene, Pabis de Melo (Carson) | University of Sao Paulo |
| Starmer, William T. (Heed) | Syracuse University |
| Steiner, William W. (Carson) | University of Illinois |
| Templeton, Alan T. (Carson) | Washington University, St. Louis |
| doVal, Francisca C. (Carson) | University of Sao Paulo |
| Wheeler, Linda (Stone) | University of Texas |
| Yoon, Jong Sik (Richardson) | University of Texas |

*The name of the senior investigator with whom the associate investigator cooperated is given in parentheses.

