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Danko Brncic and the flower-flies

Danko Brncic y las moscas de las flores

HAMPTON L. CARSON

Department of Genetics and Molecular Biology, John *A.* Burns School of Medicine, University of Hawaii at Manoa, 1960 East-West Road, Honolulu, Hawaii 96822, U.S.A., email: hampton@hawaii.edu

ABSTRACT

This is a personal essay commemorating Brncic as a keen naturalist/evolutionist in the best Darwinian sense. His innate curiosity, so important in a scientist, led him to the discovery of cenain unique drosophilid flies in Chile. The life cycle of one species of flower-fly *(Drosophila flavopilosa)* is of special interest. Females oviposit in fresh flowers of a solanaceous plant that is widespread in Chilean valleys. Twenty years of combined ecological and genetic work, carried out on this species, pioneers the use of a highly specialized *Drosophila* species in the study of population genetics and evolution. Many eco-genetic features are revealed that are important supplements to observations made on the widely-studied generalist species of the genus.

Key words: flower-breeding *Drosophila*, genetic specialization, ecology, population genetics, evolution.

RESUMEN

Es este un ensayo de naturaleza personal, celebrando a Danko Brncic como un natualista y evolucionista de la mejor calidad en la verdadera tradición darwiniana. Su curiosidad inata tan importante para el científico, lo Ilevó a descubrir algunos drosofilideos chilenos con propiedades únicas. Especial interés tiene el ciclo vital de *Drosophila flavopilosa,* especie adaptada a flores: la hembra deposita sus huevos en las flores de una solanacea ampliamente distribuida en los valles de Chile. Veinte años de investigación geno-ecológica sobre esta especie resultaron en datos pioneros, con el uso de especies altamente especializadas para aclarar cuestiones generales de genética y evolución de poblaciones. Muchos hechos importantes fueron revelados, distintos de aquellos observados en las especies generalistas y ampliamente distribuidas.

Palabras clave: *Drosophila*, especialización genética, ecología, genética de poblaciones, evolución.

INTRODUCTION

I write here to celebrate Danko Brncic's remarkable set of researches on a curious and extremely interesting flower-breeding species of *Drosophila* that he found in the valleys of Chile. I take joy in concentrating on this particular aspect of his highly productive and diversified research career. His study of the flower flies dramatically illustrates my own greatest love in science: the pursuit of biology, especially genetics, through natural history. Such studies are stimulated and initiated wholly by basic curiosity about the natural world. Brncic was rare among geneticists in being an acutely sensitive naturalist in the best Darwinian tradition. With respect to the flower-flies, his research was done with the prime desire only to know, and not necessarily to apply the findings to man's use.

The natural history of Drosophila *Brazilian style*

I first met Danko in 1951 when the two of us were visiting workers in the São Paulo laboratory, then headed by André Dreyfus. Dobzhansky had preceded us there by a few years and had left a legacy by discovering and encouraging two young Brazilian colleagues, Antonio Brito da Cunha and Crodowaldo Pavan. Their imaginative work on South American drosophilids soon became legendary. The research careers of both Danko and myself were strongly and permanently influenced by the delightful laboratory atmosphere these two created. Even more significantly, we went together on many of Pavan's adventurous field trips to various remote parts of Brazil. Another young worker who was doing cytological work and occasionally collected with us was Francisco M. Salzano from Rio Grande do Sul. Later he concentrated on human genetics, recently becoming one of the far too few South American scientists to be elected to the National Academy of Sciences of the United States.

During our stay in Brazil, Danko and I found that we shared a common interest in trying to find the natural oviposition sites that the many "wild" species of Drosophilidae were utilizing. We felt this might be a clue to discovering basic facts about the genetics and ecology of those many species that were then known only as adult specimens caught in nets or attracted to baits. The *Drosophila* species that are widely used for population genetics in Europe, North and South America and Japan tend to be generalists both in feeding and ovipositing. Thus, they are able to complete their life cycle using a wide variety of both natural and artificial substrates. These species serve the science of population genetics well since they take readily to laboratory culture. Field studies of wild *Drosophila,* however, reveal many species that do not respond well to laboratory culture. Females often refuse to oviposit on artificial media, limiting the value of the genus for the study of evolutionary biology.

At that time, none of us suspected that fresh, unfermented flowers might be important hosts for various wild drosophilids. We were aware that Sturtevant (see 1921) had described several species found in the flowers of *Datura* and *Cucumus* (melon) in tropical America. He successfully reared adults of *Drosophila florae* from *Datura* flowers collected in Costa Rica. The conventional wisdom remained, however, that drosophilids were relatively indiscriminate users of fermenting herbage, especially fruits. Few reports, if any, of strict specificity of oviposition sites had been made. We were busy in the laboratory with chromosomes of generalists and the subject of species possibly having cryptic natural oviposition sites was shelved. In 1951, Danko went to work in Dobzhansky's laboratory in New York, and I returned to St. Louis; both of us were thus temporarily isolated from most warm-climate natural breeding sites.

Collections in the middle-American tropics

A few years later, however, In 1956, 1959 and 1960, the Genetics Foundation of the University of Texas, through Marshall Wheeler and Wilson Stone, sponsored a number of *Drosophila*-collecting surveys in tropical areas in Central America and northern South America. At that time, William B. Heed was the leading field investígator for this project. He had already spent considerable research time trying to uncover specific breeding sites of tropical species. Meredith Carson and I accompanied him on these three exploratory expeditions. Marvin Wasserman joined us for the 1956 trip.

The thrust of this research, as emphasized at the time by Professor Wilson Stone, was to discover as many new species of *Drosophila* as possible! Bill Heed was an indefatigable species-finder, having collected extensively in El Salvador (Heed 1957). He led us into many interesting back-country areas in Costa Rica, Panamá and Colombia. This was terra incognita for drosophilids in those days. Heed's approach, which we all adopted, was to seek out every conceivable ecological niche from seashore to mountain-top where *Drosophila* species might be found. Bill and I used clumsy beating and sweeping nets as well as various unpleasant fermenting baits and other substances that appeared to attract adult flies.

As Pavan always did, the collections were made in forest and savanna areas having as much ecological integrity as it was possible to find. Meredith's particular contribution to the collecting was to have a few empty vials at hand and approach anything in the forest, often on hands and knees, that might have drosophilids associated with it or resting on it. She would simply imprison the fly under a small glass vial. We named this the "thumb and forefinger method" of collecting *Drosophila.* She found many species that the other methods had missed. Stone's dictum had indeed provided us with a delightful exercise in the pure joy of discovery.

The richness of the drosophilid fauna, revealed in these tropical areas, was almost incredible; notebooks were filled with preliminary notes on what appeared to be dozens of new species and new groups of species. Some have not been described even to this day.

We always carried with us standard laboratory food which was suitable for generalist species. Each wild-caught female was placed on this food in the hope that eggs and larvae would appear, yielding a successful culture. This aspect of the work was notably unsuccessful, since many, if not most, of the female specimens, appearing to be new species, did not respond by ovipositing. Dried voucher specimens for most such species were returned to the Texas laboratory. Many of these appeared to belong to new or little-studied species-groups and the fact that we could not rear them greatly complicated systematic studies as well as those of chromosome cytology, behavior and ecology. The complex itineraries of the field trips provided very little time to collect and incubate suspected oviposition substrates from which specimens might be reared out.

Our attention in these expeditions included both the lowland Tropics and the extremely rich ecosystems at intermediate altitudes (up to approximately 1200 m). In all three trips we collected drosophilids from *Datura* flowers, especially the large and spectacular species *D. arborea* (reina de la noche) but were not able to prepare and incubate samples so as to prove the presence of eggs or larvae. It never occurred to us that other Solanaceae might also be hosts of *Drosophila*. In 1956, we made short excursions into the páramos above 2000 m in Costa Rica and near Bucaramanga in Colombia. Baits produced little, and the high altitude environments were rejected as a fruitful site for drosophilid collecting.

A surerise in the páramos

Only in 1960 did we again reach the páramos; this time we went up from Santa Fé de Bogotá, Colombia to an altitude of about 3000 m. Not far from the height of the pass, we happened to notice several tall and spectacular composites in full flower; these were later identified for us as species of *Espeletia* and *Bidens*, growing along with the giant bromeliad Puya. Idly stopping to enjoy looking more closely at these magnificent plants, we started seeing new and unknown drosophilds in and around the flowers. Specimens were captured individually by Meredith's "thumb and forefinger" method. The flowers of *Bidens rubifolia* alone ultimately yielded five new species of *Drosophila* and two of *Scaptomyza*. Flowers of other *Chrysanthemum-like* composites frequently yielded interesting specimens. For the rest of our short stay at Bogotá we found 23 apparently new species of the family Drosophilidae in flowers. With the help of Professor Jesús M. Odrobo of the Instituto de Ciencias Naturales, Universidad Nacional de Colombia, we identified 16 species of host plants, belonging not only to Compositae but also to seven other plant families. None of these *Drosophila* species could be cultured. The complete life cycle of six species, however, was determined either by viewing oviposition, finding larvae, or rearing adults directly from flowers collected in nature (Heed et al. 1961).

This "fly/flower ecosystem" appeared to be by no means confined to the páramos: examples were uncovered in plant flowers from areas as low as 1,500 m. Although it had yet to be proved, these observations suggested that many of these flies tended towards strict host-plant specificity. This implied that oviposition itself might also be highly host-specific, at least in some cases.

Before leaving Bogotá, we visited the laboratory of Professor Alice Hunter at the Universidad de los Andes, who showed us some *obscura* group drosophilids she had been catching at various places on the Llano Alto. We were able to confirm that these were indeed *Drosophila pseudoobscura*, previously unknown in South America. At the same time we told her of the apparent *Drosophila* flower ecosystem.

A study in depth: the role of Danko Brncic

At about the same time as the above events, Danko Brncic had independently discovered, near Santiago, Chile, an intimate ecological relationship between a widespread Chilean species, *Drosophila flavopilosa*, and the solanaceous plant *Cestrum parqui*. This discovery is first mentioned in Wheeler et al. (1962), in which the first of Danko's photographs were published. Alone among *Drosophila* workers, Danko soon focussed intense scrutiny on this case where the species appeared to carry strict adaptational features relating to breeding on flowers. The result was the unfolding of an extensive, truly classical study of eco-genetic details that are uniquely interesting and were described in detail for the first time (Brncic 1962, 1966, 1970, 1983).

Drosophila flavopilosa is closely adapted to utilize the flowers of this specific host plant and cannot be reared on laboratory media. Using the spines on the ovipositor, the female scarifies the fresh young flower at a particular point and then oviposits. A single egg is laid per flower; each flower thus harboring a maximum of one larva and one puparium, yielding only a single adult specimen. Ovipsition only on this plant species reflects the specificity of the relationship. Both fly and plant are distributed in many Chilean valleys from near sea level to about 1700 m altitude and appears to be accompanied everywhere by this same *Drosophila* species.

A particular genetic attraction of this species is the fact that once the ecological niche was discovered, mature larvae could easily be found developing in the flowers. When their salivary glands were examined microscopically, they yielded a splendid set of favorable polytene chromosomes, complete with a number of balanced inversions varying in frequency form one population to the next. In exploiting this unique material, many instances of geographical, altitudinal and seasonal shifts in genetic structure were found by Brncic, showing how closely the genetic material of the species was tracking altitudinal, geographical and other environmental variables. This work had the unique dimension of being done on specimens captured without the use of baits or other artificial and ecologically imprecise methods for obtaining specimens. This greatly enhanced the value of the genetic data as a reflection of natural ecological situations. A valuable case of natural parasitism of the fly larvae by two species of wasps was also discovered during this work.

These research possibilities were exploited by Brncic over about 20 years. The research integrates, in a unique way. two major sciences, genetics and ecology. This has had the effect of stimulating others to study flower/*Drosophila* ecosystems in various parts of the world, including, for example, Panamá (Pipkin et al. 1966), Hawaii (Heed 1968, Montague 1984), New Guinea (Okada & Carson 1980, 1982), Africa (Lachaise et al. 1982), Brazil (Vilela 1984), and Australia (Cook et al. 1977, Starmer et al. 1998). In Indonesia, Sultana et al. (1999) report further detailed investigations of *D. elegans,* a flower specialist closely related to the famous generalist, *D. melanogaster.*

Boreal forests of the northern hemisphere, such as are found in northern North America, Europe and Japan harbor a substantial number of species of *Drosophila.* The great majority of these species are attracted to fermenting baits and are reasonably easy to culture in the laboratory. Accordingly, these species are well-known to geneticists. The members of this fauna, however, almost without exception, appear to feed and breed on naturally occurring fermenting substances such as fungi, sap fluxes of trees, small fruits and berries, decaying or fermenting leaves and, to a small extent, decaying flowers or flower parts (Carson & Stalker 1951, Basden 1954, Kimura et al. 1977, Shorrocks 1982). Certain species are nevertheless clearly differentially adapted to favor, for both feeding and oviposition, certain types of fermenting substrates over others. Groups or assemblages of species breed on mushrooms, others favor slime fluxes and still others small berries. Almost none, however, are strict specialists. Although exceptions may yet be found, we must conclude that no species or species-group in the boreal fauna appears to demonstrate specific oviposition on the flowers of a single specialized niche as in the case of *D. flavopilosa.*

Ecological genetics

As I have stated previously, my intention in this essay is not to review the detailed contents of Brncic's fine work but rather to emphasize the investigative spirit in which it was carried out. When I was a beginning faculty member at Washington University, St. Louis in 1943, I was keen to study microevolution genetically. I had arrived there as someone trained in cytological chromosome work on specimens that had been obtained from a mycetophilid fly species *(Sciara impatiens).* This species could be obtained only inside of glass greenhouses. Shortly I became aware of the need for the integration of the genetic and ecological approaches to evolution. This came about particularly through association with Professor Edgar Anderson, a prominent member of the botanical faculty at Washington University.

Anderson was then offering an innovative course of study which he had boldly entitled: "Genetics and natural history". He had little patience with research on flies like *Sciara,* or indeed *D. melanogaster,* the ecology of which appeared to exist only within glass bottles in the laboratory.

"To discover principles of the evolutionary process" Anderson would say, "you must apply your genetic studies directly to your organism in its true ecological setting. In your case, this means study of the mouldy places under the benches of greenhouses or, if you must study *Drosophila melanogaster,* you need to find out exactly what they are doing around garbage bins". As new instructors, the late Harrison Stalker and myself informally enrolled in Edgar's course. The field trips to the limestone glades of Missouri in particular stand out in my mind. He urged us to make simple observations of natural ecological situations, especially those biological events related to plant reproduction. His favorite topic was the recognition of how natural hybridization (a genetic property), may frequently be found in areas where a "hybridized" environment occurs (an ecological property). Stalker and I were both skeptical.

Another interesting Andersonian dictum was that the field investigator should not be over-organized or in a hurry to complete the work and publish quickly in order to impress an administrative officer who supplies funds. A student of his told me that he had once planned a dense and complicated set of field manipulations on a species of plant confined to the limestone glades on which he had chosen to work. Anderson's view was that such a super-organized approach was a sort of "busy-work" that hinders the exercise of basic curiosity and therefore may inhibit the finding of something new. He advised the student to go to the study area and be "laid back". It was specifically mandated that the investigator should sit in the forest or lie down in the grass and decompress his mind for at least a half hour, just idly observing the clouds going by in the sky and with no preconception as to what he might see that was interesting. The student reported that he had more or less obeyed Anderson, but nevertheless had secretly dismissed the enforced idleness a "bunch of nonsense". Later, however, he changed his mind, reporting that, one day, while sitting there eating his sandwich lunch, he "just happened to notice" something unexpected about the microhabitat of his plant that had changed his perspective in an important way.

The study of the flower-flies, of course, constitutes only a small portion of Brncic's life work. An overview reveals many fascinating and valuable contributions on diverse topics in genetics and evolution. In several cases, these are important basic contributions to the genetics of populations. One example is his classic paper of 1954 that shows how gene recombination can cause breakdown of the genetic basis of the adaptive responses of a specific local population. The inference from this finding is that each local sexual population has a unique and complex genetic structure maintained by selection in nature. What could be more fundamental than this? During the 1980's and 90's, 40 years later, he took the lead in gathering timely data documenting the genetic aspects of the extraordinary case of the recent invasion of western North and South America by the European species *Drosophila subobscura* (see Brncic 1995).

A speculation and a conclusion

Many of the most innovative biological scientists have naturally had a relaxed approach to their work that has made novel discoveries possible. I have mentioned Pavan, Heed and Anderson in this connection. Even if none of us knows for sure how Danko, the naturalist, discovered the flower flies, I would like to speculate that the following happened on one day. He may have been sitting eating his lunch in the neighborhood of the *Cestrum* plants on a Chilean hillside when he, acting as Anderson would predict, "just happened to notice" the slight bulge in a single flower. On inspection, it may have yielded the first single crucial *Drosophila* larva, carrying chromosomes waiting to be found. As teachers of science in general, and biology in particular, the contemplation of Danko Brncic's dedication to pure science might be used to help us encourage and glorify the pursuit of basic curiosity among our students.

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