

AN ANOMALOUS HYBRID ZONE IN *DROSOPHILA*

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Abstract.—Despite the genetic tractability of many of *Drosophila* species, the genus has few examples of the “classic” type of hybrid zone, in which the ranges of two species overlap with a gradual transition from one species to another through an area where hybrids are produced. Here we describe a classic hybrid zone in *Drosophila* that involves two sister species, *Drosophila yakuba* and *D. santomea*, on the island of São Tomé. Our transect of this zone has yielded several surprising and anomalous findings. First, we detected the presence of an additional hybrid zone largely outside the range of both parental species. This phenomenon is, to our knowledge, unique among animals. Second, the genetic analysis using diagnostic molecular markers of the flies collected in this anomalous hybrid zone indicates that nearly all hybrid males are F₁s that carry the *D. santomea* X chromosome. This F₁ genotype is much more difficult to produce in the laboratory compared to the genotype from the reciprocal cross, showing that sexual isolation as seen in the laboratory is insufficient to explain the genotypes of hybrids found in the wild. Third, there is a puzzling absence of hybrid females. We suggest several tentative explanations for the anomalies associated with this hybrid zone, but for the present they remain a mystery.

Key words.—Ecological adaptation, hybrid swarm, hybridization, reproductive isolation, speciation.

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Although *Drosophila* has been a premier object of evolutionary studies, including studies of speciation, there has been a notable lack of work in the genus on one important phenomenon: hybrid zones. Despite the fact that related species can often be crossed in the laboratory, facilitating genetic analysis of character differences or reproductive isolating barriers, few studies have reported instances of hybridization in the wild (Gupta et al. 1980; Lachaise et al. 2000). Among these, most produce sterile or inviable offspring (so that no introgression between species is possible), while nearly all others are one-time or sporadic instances of hybridization between sympatric species (e.g., Carson et al. 1989).

Thus, despite its genetic tractability, *Drosophila* lacks the “classic” type of hybrid zone in which two species’ ranges overlap, with a gradual transition from one species to another through an area where hybrids are found (see Barton and Hewitt 1981; Harrison 1993; Arnold 1997; Coyne and Orr 2004). Such hybrid zones offer several windows on speciation, including estimating the number of genes involved in hybrid unfitness, determining gene- and chromosome-specific patterns of introgression, and investigating whether hybrids have superior fitness in nature. Occasionally, hybridization between individuals of different species may promote the origin of new genetic adaptations or even new species, a phenomenon extensively studied in plants (Grant 1981; Rieseberg 1997; Rieseberg et al. 1995).

Drosophila yakuba is a mainland African species, widespread throughout sub-Saharan Africa from Senegal to South Africa. The species occupies a diversity of more or less open habitats including semiarid areas, periferest lowland savannas, montane grassland, *Brachystegia* woodland, pioneer Okume forests, secondary forests, and semidomestic habitats (notably coffee and cacao plantations), but it is absent in rainforests. *Drosophila yakuba* has also extended its range to

neighboring islands, including Madagascar and Zanzibar in the Western Indian Ocean, and all the Gulf of Guinea islands in the Eastern Atlantic Ocean (i.e., Bioko, Príncipe, São Tomé, and Annobon).

In contrast, *D. santomea*, discovered in 1998, is endemic to São Tomé, a 860 km² volcanic island 255 km off the coast of Gabon (Lachaise et al. 2000), which, as noted, also harbors *D. yakuba*. On the slopes of the highest volcano, Pico de São Tomé (el. 2024 m), *D. yakuba* lives at elevations below 1450 m and *D. santomea* at elevations above 1150 m. Between these elevations the species ranges overlap, forming a hybrid zone in which one finds a low frequency (about 1%) of hybrids (Lachaise et al. 2000; this study). In São Tomé, *D. yakuba* is found largely in towns, disturbed sites, cutover areas, open plantations, and edges of the rainforest, whereas *D. santomea* lives only in montane rain and mist forest. We suspect that *D. santomea* breeds largely, if not exclusively, in figs of the endemic subspecies *Ficus chlamydocarpa fernandesiana* (see Appendix).

The species show substantial sexual isolation when tested in the laboratory (Coyne et al. 2002, 2005; Llopart et al. 2002), with the two interspecific matings occurring less frequently than intraspecific matings, and the mating between *D. santomea* females and *D. yakuba* males occurring very rarely. The pair fails to show any evidence of “reinforcement,” that is, any increase in sexual isolation between species in the hybrid zone (Coyne et al. 2002). Population-genetic analysis of numerous loci show that there is some introgression between these species, though it is not extensive, limited mainly to mtDNA and two nuclear regions (Llopart et al. 2005). Molecular evidence puts the divergence between *D. yakuba* and *D. santomea* at about 400,000 years ago (Llopart et al. 2002). They are sister species, more closely related to each other than to any other species in the *D. melanogaster*

subgroup. It is likely that *D. santomea* descends from representatives of the ancestral lineage of *D. yakuba* that colonized the island at that time, and that the present contact between *D. santomea* and *D. yakuba* resulted from secondary colonization by the latter species, probably during the last 500 years, when the colonizing Portuguese turned large sections of rainforest along the coast into plantations (Llopart et al. 2005). This scenario is supported by the present distribution of the species: their ranges overlap at ecotones between the cultivated areas at lower elevation and primary rainforest at higher altitudes (see below).

Here we describe a rare classic hybrid zone in *Drosophila*, between *D. yakuba* and *D. santomea* on São Tomé. Although areas of hybridization undoubtedly exist elsewhere on the island, we conducted a transect through one that was long and accessible, extending from sea level to the top of the highest volcanic mountain, Pico de São Tomé, at 2024 m. (A detailed description of the geography and vegetation of the transect as well as data on *D. yakuba* and *D. santomea* breeding sites can be found in the Appendix.) Our study of this zone has yielded several surprising and anomalous findings, including unexpected genotypes of hybrids and the presence of an additional hybrid zone largely outside the range of both parental species. While the latter phenomenon is, to our knowledge, unique among animals, the former finding teaches us a cautionary lesson important in evolutionary studies: patterns of sexual isolation and hybridization can be strikingly different in the laboratory and in the field.

MATERIALS AND METHODS

Fly Collections

We collected flies on the island of São Tomé during several field trips over an eight-year period (1998–2005). We concentrated on a northeast-southwest transect that started at sea level, at a garden outside São Tomé City, proceeded toward higher elevation through cultivated fields, continued through the rainforest, up to the summit of Pico de São Tomé (2024 m), and then 300 m down the other side of the Pico, with two collecting stations on the southwestern slope of this side at 1850 and 1700 m respectively (see Appendix). For each collection site we recorded the number of *D. yakuba*, *D. santomea*, and potential hybrid flies, as well as the elevation (Table 1). During April 1998, February 2001, and January 2003 we collected most of the flies at 1566 m and below. In March 2003, we sampled the rainforest region above 1566 m on Pico de São Tomé and discovered, unexpectedly, a large number of hybrid males. We verified this unusual result by making three more collections during June 2003, January 2004, and January 2005, all of which consisted largely or completely of hybrids at high elevations. Thus, the anomaly of a high-altitude area harboring hybrids almost exclusively—an area above the altitudinal range of the parental species *D. yakuba*—was observable over three years and during both wet and dry seasons of one year. At all stations, the species composition of collected flies was consistent through the different years, as was the relative abundance of *D. yakuba* and *D. santomea* for a given elevation; therefore, we pooled our data among different years. Most captures were carried out using baits of mashed banana in traps made from 1-L plastic

TABLE 1. Number of *Drosophila yakuba*, *D. santomea*, and hybrids on a northeast–southwest transect of São Tomé Island. The “classic” hybrid zone separating the two parental species and the “anomalous” hybrid zone are indicated in bold. W indicates collecting stations on the far (west) side of the peak.

Elevation (m)	<i>D. santomea</i>	<i>D. yakuba</i>	Hybrid males
0	0	108	0
520	0	148	0
700	0	38	0
880	0	133	0
955	0	52	0
1060	0	54	0
1153	281	184	0
1186	75	81	0
1200	306	53	2
1210	21	40	0
1235	11	45	0
1250	126	66	0
1290	27	5	0
1300	8	1	0
1320	45	6	1
1340	17	5	1
1350	16	0	0
1360	7	0	1
1400	25	1	1
1415	44	1	0
1430	157	1	0
1435	4	0	0
1440	62	1	0
1450	46	0	0
1455	89	0	0
1470	8	0	0
1482	42	2	1
1495	11	0	0
1500	17	0	1
1540	3	0	1
1550	6	0	1
1566	36	0	1
1600	17	0	0
1700	2	0	0
1800	3	0	0
1900	2	0	19
1930	1	0	15
1950	0	0	1
1980	1	0	4
2024	1	0	25
W1850	0	0	1
W1700	0	0	0

bottles suspended from the vegetation at eye level, although in 2005 we also collected by sweeping over mashed banana bait that had been spread on the ground.

Molecular Markers and Genotyping

Upon collection, we preserved the specimens in absolute ethanol and, once at the laboratory, extracted DNA from single flies using the Puregene DNA Isolation Kit for paraffin-embedded tissue (Gentra Systems, Minneapolis, MN). To identify hybrids between *D. yakuba* and *D. santomea*, we used pigmentation patterns as a crude first approach in the field (Llopart et al. 2002) and confirmed this using diagnostic molecular markers based on restriction fragment length polymorphisms (RFLP). These same markers were also used in a random sample of females to investigate the presence of hybrid females in our collections. Using sequence data obtained in a previous study (Llopart et al. 2005), we designed

six diagnostic molecular markers, one on each chromosome arm including the dot (fourth) chromosome. A diagnostic molecular marker is defined as a single nucleotide difference affecting a restriction endonuclease (RE) site that is fixed between *D. yakuba* and *D. santomea*. The diagnostic regions were amplified by polymerase chain reaction (PCR) using approximately 25 ng of DNA. PCR products were purified using the Wizard MagneSil PCR clean-up system (Promega Corp., Madison, WI) and incubated overnight at 37°C using 10–20 units of the RE. Digested PCR products were visualized in 2% agarose gels stained with ethidium bromide. The markers and REs used are: *rux* (assayed with *Bgl*III) on the X chromosome, *Rpl27A* (assayed with *Eco*RI) on 2L, *Ngp* (assayed with *Hae*III) on 2R, *Lsp1-γ* (assayed with *Acc*I) on 3L, *krz* (assayed with *Rsa*I) on 3R, and *ci* (assayed with *Hpa*I) on the fourth chromosome.

RESULTS

We collected 2618 flies from 51 different sites: 1022 *D. yakuba*, 1492 *D. santomea*, and 104 flies (97 males and 7 females) diagnosed as possible hybrids based on morphology (i.e., flies with an intermediate degree of abdominal pigmentation). To determine whether these ‘‘hybrids’’ identified by morphological intermediacy were genuine, and to ascertain whether they were F₁ or later-generation hybrids, we genotyped them at six diagnostic markers. To investigate the presence of hybrid females in the collections, these same markers were also used to genotype 40 additional females, 34 of them collected at the same sites where most hybrid males were detected. Our results indicate that only 76 of 97 males are bona fide hybrids (i.e., heterozygous for *D. yakuba* and *D. santomea* markers at one or more diagnostic loci), and that most of these (73/76) are F₁ hybrids. (Despite some errors of identification, these results do show that we are able to identify most hybrids accurately.) We also found three hybrids probably produced by F₁ hybrid females backcrossed to *D. yakuba* males, two heterozygous at three of five autosomal markers and one heterozygous at two markers. (In the laboratory, F₁ hybrid males are sterile and, thus, individuals of mixed genotype must be generated in backcrosses.) The remaining males are homozygous at all markers, three with the *D. yakuba* allele and 18 with the *D. santomea* allele. Surprisingly, we detected no hybrid females. Assignment of all 144 genotyped flies into the F₁, backcross, and parental classes was confirmed by a Monte Carlo–based method to identify hybrids (Anderson and Thompson 2002).

The distribution of hybrid males among the different collection sites is extremely anomalous. Only six of the 76 individuals were captured in the zone of overlap between the two parental species (1150–1450 m elevation). The low frequency of hybrids in this overlapping area (6/1774) contrasts with the extreme abundance of hybrids found above 1500 m (48.6%, 68 hybrid males, 39 *D. santomea* females and 33 *D. santomea* males). As shown in Table 1, the majority of hybrids are present in a zone between 1900 and 2024 m, an area where no *D. yakuba* was ever collected, and at many collecting stations where we captured no *D. santomea* either. The presence of hybrids at high altitude is not a one-time anomaly, but was observed over the course of three years

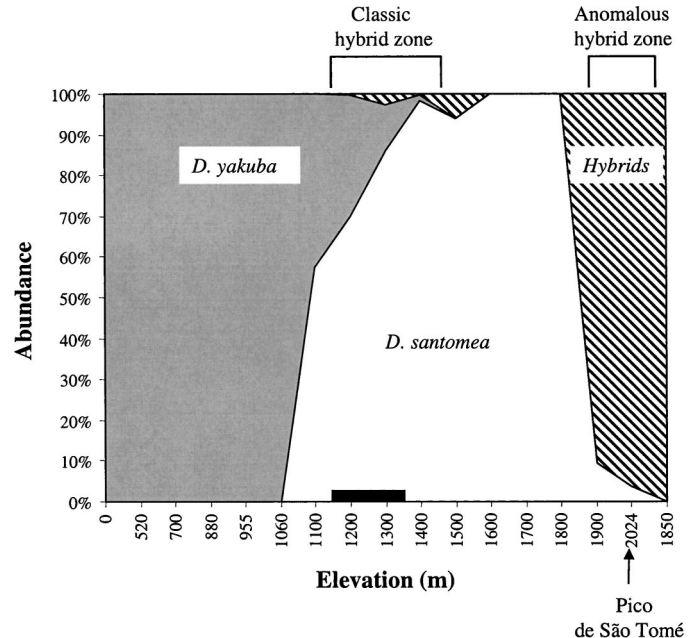


FIG. 1. Relative abundance of *Drosophila yakuba*, *D. santomea*, and hybrid flies at different elevations. The horizontal filled box marks the gradual transition between open cultivated fields/secondary forest and primary/rain forest (from Bom Sucesso at 1150 m to the Obo Natural Park entrance at 1350 m). The figure was constructed pooling the data of Table 1 for intervals of 100 m.

and four collections, as well as during both wet and dry seasons. The elevations at which hybrids were collected lay outside the range of either *D. yakuba* or *D. santomea* (non-parametric Mann-Whitney *U* test, $P < 0.0001$ in both cases). Thus, it seems that along this transect the species have two hybrid zones, one in the area of overlap between the ranges of *D. yakuba* and *D. santomea* and the other at higher elevation, far from the area of distribution of *D. yakuba* (Fig. 1). We will refer to the lower-elevation area of overlap between the two species as the *classic* hybrid zone and to the higher altitude area as the *anomalous* zone.

Another striking observation is the lack of hybrid females in our collections, a finding that deserves careful consideration. The small number of morphologically diagnosed hybrid females (four and three collected in the classic and anomalous hybrid zones, respectively) could be interpreted as a failure to recognize hybrid females in the field. Although the intermediate pigmentation pattern shown by F₁ hybrid females in the laboratory is quite distinct (Llopart et al. 2002), all seven females diagnosed as hybrids based on their morphology were actually homozygous for *D. santomea* alleles at all six markers, indicating that these flies are neither F₁s nor backcrosses. To further investigate the possibility of undetected hybrid females in the wild, and specifically in the anomalous hybrid zone where there is a striking abundance of hybrid males, we genotyped 34 females collected above the classic hybrid zone. We also genotyped six additional females collected in the classic hybrid zone. We did not find any *D. yakuba* alleles among these females for any of the molecular markers. This suggests an absence of hybrid females in our collections, hence in the wild, because we de-

terminated the genotype of every female collected above 1540 m. Thus, the anomalous hybrid zone appears to be occupied by hybrid males but not by hybrid females.

The genetic analysis of the 76 detected hybrid males shows that, based on the heterozygosity at all five of the autosomal markers, 73 are F_1 hybrids. Moreover all of these F_1 hybrids had the X chromosome of *D. santomea*. These F_1 hybrid males must therefore have been produced by matings between *D. santomea* females and *D. yakuba* males. (In the laboratory, F_1 hybrid males between *D. yakuba* and *D. santomea* are completely sterile, and we have limited evidence that suggest that in the wild this is also the case. One of the F_1 hybrid males collected on the east slope of our transect, at 1900 m, was successfully brought alive to the laboratory, mated with *D. yakuba* and *D. santomea* virgin females, and later dissected. This dissection revealed spermless testes, and no offspring were ever obtained despite the observation of copulations with four females.) The remaining three hybrid males caught in nature, two collected in the anomalous hybrid zone (1900 m) and one in the classic hybrid zone (1200 m), were probably produced by backcrosses of F_1 hybrid females to *D. yakuba* males. There are three shared features among these backcross flies: the marker on the X chromosome shows the *D. santomea* allele, the marker on the fourth chromosome is heterozygous, and the marker on chromosome 2R is homozygous for the *D. yakuba* allele. This last feature is consistent with a previous study indicating the failure of chromosome arm 2R to introgress between *D. yakuba* and *D. santomea* (Llopart et al. 2005). Of course, the study of more hybrids is required to ascertain whether these common genotypes are simply coincidental, or represent a genotype that can exist in the wild because it has a combination of "permissibly introgressable" genes that does not cause inviability.

DISCUSSION

The hybrid zone formed by *D. yakuba* and *D. santomea* in São Tomé appears to be quite unique among animals for several reasons. First, the location of the "upper" hybrid zone is anomalous because the vast majority of hybrids are found not where the ranges of the two parental species overlap, but in a territory where *D. santomea* occurs only sparsely and *D. yakuba* is completely absent. We originally suspected that the parental *D. yakuba* might be found on the southwestern slope of Pico de São Tomé, as this slope is drier and perhaps a more suitable habitat for *D. yakuba*. However, despite the fact that our sampling of the southwestern slope was limited (we had only two collecting stations), we collected one F_1 hybrid male but no *D. yakuba*. The unusual location of the upper hybrid zone is mysterious.

Scriber and Ordling (2005) have recently discovered a population of tiger swallowtail butterflies from the Battenkill River Valley (at the New York/Vermont border) that shows life-history traits and allozyme frequencies intermediate between *Papilio canadensis* and *P. glaucus*. This hybrid swarm has resulted from rapid introgression and occupies a unique thermal niche along the cooler edge of the traditional hybrid zone. This is not the case in the *D. yakuba*–*D. santomea* anomalous hybrid zone because the inhabitants of the hybrid zone clearly do not form an interbreeding hybrid swarm that

has found a unique ecological niche at high altitude: we not only failed to collect females, but the vast majority of males we collected were F_1 hybrids and therefore were sterile. Sterile hybrids and an absence of females cannot of course constitute a hybrid swarm. Thus, the anomalous hybrid zone does not conform to the classical definition of a hybrid swarm proposed by Harrison (1993; pg. 6) as "a diverse array of recombinant types."

These hybrids must be generated by repeated crossing between the parental species, although we do not know where this takes place. The definition of a bimodal hybrid zone (Jiggins and Mallet 2000), a zone containing individuals genetically similar to one or other parental genotype, with few intermediates, does not apply either. Again, the collection of individuals from backcrosses of hybrids to *D. yakuba* above 1500 m, in a zone clearly lacking *D. yakuba*, is puzzling (where do the *D. yakuba* involved in the backcross come from?). A more plausible hypothesis, but one that still strains credibility, is that F_1 hybrid males are formed at lower elevations, in the classic hybrid zone, and then migrate to higher elevations. This migration might be due to either behavioral anomalies of these hybrids or perhaps their preference for cooler temperatures. The novel genotype of hybrids may confer on them a unique ecological tolerance or preference beyond the range of the parental species, as is seen in several plant hybrids (Rieseberg et al. 2003; Coyne and Orr 2004, ch. 9) and recently reported in a new diploid hybrid species of the tephritid fly genus *Rhagoletis* (Schwarz et al. 2005). Other scenarios are also possible. For instance, there may be several humid corridors on the southwestern side of the island at high elevation, and these may support more east-slope vegetation and serve as dispersal routes for hybrid males. Alternatively, on the wet slope at lower elevations, there may be much more interdigitation of vegetation types between drainages and adjacent drier ridge topographies, which could change the context for geographic contact on that side of the Pico. Thus the anomalous hybrid zone could show a mosaic-type pattern. Obviously, we need more studies on both the ecological differences between both hybrid zones, especially on the microgeography, and the behavior of hybrids in the laboratory.

Second, there is a remarkable absence of any hybrid females (F_1 or backcross) in our collections, both in the classic and anomalous hybrid zones. This is, of course, in strong contrast to the abundance of F_1 hybrid males. The absence of hybrid females cannot reflect their innate inviability, because, at least in the laboratory, they are produced in equal proportions to hybrid male offspring from the cross of *D. santomea* females to *D. yakuba* males (Coyne et al. 2004). One could argue, perhaps, that hybrid females are either not attracted to banana baits or not able to enter the traps because of behavioral anomalies. (However, this seems quite unlikely because both parental species and hybrid males were collected in this way.) We tested this directly by conducting laboratory release-and-recapture experiments using hybrid offspring of crosses between *D. santomea* females and *D. yakuba* males. These experiments show that F_1 hybrid females are attracted to hanging banana-bait traps at least as readily as hybrid males. Our results thus suggest that F_1 hybrid females may show environmentally dependent inviability, a

form of extrinsic postzygotic isolation (Coyne and Orr 2004). The lack of F_1 hybrid females in our collections also explains the scarcity of backcross flies, as the latter must be produced by F_1 females.

Finally, all F_1 hybrid males in our collections are the offspring of *D. santomea* females and *D. yakuba* males. This is, of course, at odds with the introgression of the *D. yakuba* mtDNA into *D. santomea* (Llopart et al. 2005). This discrepancy could be explained, at least partially, by a difference in abundance between *D. santomea* and *D. yakuba* in the hybrid zones, the “Wirtz effect” (Wirtz 1999; Chan and Levin 2005), with *D. yakuba* being the less abundant species shortly after its colonization of São Tomé. If the species occur in unequal proportions, and there is already mate discrimination between them, then there should be more hybridization between rare females and common males than vice versa. This gave the *D. yakuba* mtDNA the opportunity to introgress into *D. santomea* shortly after the invasion of São Tomé by *D. yakuba*, and to be a possible target of Darwinian natural selection.

The observation that all our F_1 hybrid males collected in the wild carry the X chromosome of *D. santomea* is, again, contrary to what we observe in the laboratory, where sexual isolation is strongly asymmetrical, with one interspecific cross, *D. yakuba* females \times *D. santomea* males, proceeding much more readily than the reciprocal cross (Coyne et al. 2002, 2005). This asymmetry is due largely to the rejection by *D. santomea* females of persistently courting *D. yakuba* males (Coyne et al. 2005). It is worth noting, however, that there is a possible ascertainment bias here: F_1 hybrid males with the *D. yakuba* X chromosome are more similar to pure *D. yakuba* males than reciprocal hybrid males are to pure *D. santomea* males. This ascertainment bias might account in part for the absence of F_1 hybrid males from the cross between *D. yakuba* females and *D. santomea* males in the classic hybrid zone, but cannot account for their absence in the anomalous zone, where we determined the genotype of every fly captured.

However, as reported in the companion paper (Coyne et al. 2005), the discrimination on the part of *D. santomea* females is much stronger than that by *D. yakuba* females, and we were unable to change this strong mating asymmetry by manipulating either the conditions of mate choice, the relative frequency of the two species, or various environmental variables. Thus, the nature of sexual isolation seen in the laboratory is unable to account for the genotypes of hybrids seen in the wild. Perhaps there are sex-specific differences in ecology that facilitate encounters between *D. santomea* females and *D. yakuba* males, or perhaps F_1 hybrid males carrying the *D. yakuba* X chromosome show reduced viability in the wild. Our results further stress the need to take into account ecological and behavioral conditions in the wild to study reproductive isolation. For the present, the anomalous hybrid zone and the particular genotypes of hybrids produced defy explanation.

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APPENDIX

Vegetation, geography and breeding sites of our transect from sea level to Bom Sucesso and the Pico de São Tomé (adapted and updated after Excell 1944; Monod 1960; White 1986, pp. 278–279; Le Joly 1995; new records from the authors).

The botanical altitudinal zonation of São Tomé Island was described by Excell (1944), Monod (1960), and White (1986), although there are some discrepancies between these accounts. Excell (1944) proposed the following zonation: lower rain-forest region (0–800 m); mountain rain-forest region (800–1400 m), described by Monod (1960) as a transition forest; and mist-forest region (1400–2024 m). In 1960, Monod stressed that the lowland rain forest was almost completely destroyed (except in some areas of southern São Tomé) and that altitudes of interzone transitions were only rough estimates. More recently, Le Joly (1995) provided much detailed botanical information on the area of our transect, especially from Bom Sucesso to the summit.

After six field trips in São Tomé montane forests, we concur with Monod (1960) that the altitudinal zonation of forests differs greatly between the northern and southern mountain blocks, and there is a considerable effect of slope direction. Accordingly, no general zonation applies for the overall island. We therefore focus here on the eastern slope of the northern mountain range, where the *Drosophila yakuba*–*D. santomea* hybrid zone has mostly been studied.

Lower Rain-Forest Region (0–800 m) *Sensu* Excell (1944)

A lower secondary forest region including mostly plantations has almost fully replaced the primeval lower-elevation rain forest (Monod 1960; White 1986) formerly ranging from 0 to 800 m above sea level (Excell 1944) and now extending to nearly 1200 m due to accelerated human encroachment upon the middle submontane forest (notably between 1998 and the present). The gently sloping forest existing at some areas of this altitude includes a diversity of genera, notably *Allophylus*, *Anisophyllea*, *Artocarpus*, *Ceiba*, *Celtis*, *Chlorophora*, *Chrysophyllum*, *Cola*, *Cynometra*, *Dacryodes*, *Dialium*, *Heisteria*, *Musanga*, and *Treculia* (Excell 1944; White 1986), intermingled with coffee and cacao and, above Bom Sucesso, tomato and cabbage plantations. This lower secondary forest region makes a typical mosaic habitat suitable for *D. yakuba*.

Mountain Rain-Forest Region (800–1400 m) *Sensu* Excell (1944)

The submontane forest between 800 and 1400 m in elevation is characterized by a remarkable diversity of Rubiaceae and Euphorbiaceae, accompanied by numerous Melastomataceae and Begoniaceae. Among Rubiaceae, no less than 24 taxa (about 41%), are endemic (Le Joly 1995). Portions of this forest are cultivated, especially between 800 and 1200 m. The characteristic trees include the Rubiaceae *Craterispermum montanum*, endemic to Gulf of Guinea islands, the Euphorbiaceae *Discoclaoxylon occidentale*; *Pseudogrostistachys africana*; *Sapium ellipticum*, the former endemic to São Tomé; and also *Maesa lanceolata* (Myrsinaceae), *Olea capensis*

(Oleaceae), *Symphonia globulifera* (Clusiaceae), *Tabernaemontana stenosisiphon* (endemic, Apocynaceae), and *Trichilia grandifolia* (endemic, Meliaceae). The current altered midelevation forest shows intermingled patterns of shrinking remnants of the mountain rain forest region and introduced trees and plantations. It is a typical transition forest with a mosaic of open and heavily forested areas. This probably accounts for the coexistence of *D. yakuba* and *D. santomea* in the upper part of that zone (between 1100 and 1450 m) and the presence of some hybrids in this area

Mist-Forest Region (1400–2024 m) *Sensu* Excell (1944)

Above 1400 m, the mist forest is easily recognizable by the endemic giant fern *Cyathea manniana* (Cyatheaceae), a diversity of endemic *Begonia* (especially the giant endemic *Begonia baccata*), and a large number of epiphytes (bryophytes, ferns, *Usnea*, Orchidaceae, *Peperomia*) covering the upland trees. The main trees are *Balthasaria mannii* (endemic, Theaceae), *Cassipourea gummiflua* (Rhizophoraceae), *Peddia thomensis* (endemic, Thymelaeaceae), *Prunus africana* (Rosaceae), and *Nuxia congesta* (Loganiaceae) (White 1986). One of the Rubiaceae, *Craterispermum montanum*, found also below 1400 m, typifies the lower tree stratum between the altitudes of 1350 and 1900 m. From 1350 to 1600 m, the northern mist forest is also characterized by two common upland endemic trees, *Tabernaemontana stenosisiphon* (Apocynaceae) and *Homalium henriquesii* (Flacourtiaceae). This is the typical habitat of the endemic montane *D. santomea*.

In the upper mist submontane forest, the summit ridge (1750–2024 m) is covered by a mist forest with smaller trees, including *Schefflera mannii* (Araliaceae), *Podocarpus mannii* (Podocarpaceae), and *Syzygium guineense* subspecies *bamendae* (Myrtaceae). In rare open areas there are occasional patches of endemic nonforest species, notably the heather *Erica* (*Philippia*) *thomensis* (Ericaceae) and the *Lobelia barnsii* (Campanulaceae), both closely related to species found in more open areas of high plateaus in Cameroon (White 1986).

Breeding sites of *D. yakuba* and *D. santomea*

Along our transect from sea level to Bom Sucesso and Pico de São Tomé we collected seven different fruits/flowers that could be potential breeding substrates of *D. yakuba* and *D. santomea*: *Artocarpus heterophyllus* (600 m), *Ficus thonningii* (1160 m), *Costus giganteus* (Zingiberaceae; 1200 m), *Ficus chlamydocarpa fernesiana* (1200 and 1500 m), *Anthocleista vogelii* (Longaniaceae; 1530 m), *Craterispermum montanum* (Rubiaceae; 1566 m) and *Palissotis* species (Commelinaceae; 1566 m). *Drosophila yakuba* emerged only from fruits of *A. heterophyllus* and figs of *F. thonningii* reared in the laboratory, but not from other substrates that successfully sustained larvae of other *Drosophila* species such as *D. nikananu* (obtained from *F. chlamydocarpa fernesiana*, and *A. vogelii*), *D. alladian* (from *F. thonningii* and *F. chlamydocarpa fernesiana*), *D. ananassae* (from *F. chlamydocarpa fernesiana*), and *D. chauvacae* (from *A. vogelii*). In the lowlands, *D. yakuba* was also extremely abundant in areas of fallen syconia of *Ficus mucus* (Moraceae).

In contrast, *D. santomea* emerged exclusively from figs of the great endemic hemiepiphytic fig *F. chlamydocarpa fernesiana*, which is widespread and abundant in São Tomé from 1200 to 1750 m in elevation. *Drosophila santomea* has been invariably and solely collected on patches of fallen syconia of this fig, and has not yet been reported on any other resources, including other species of *Ficus* such as *F. thonningii*, which grows on São Tomé up to 1450 m elevation, or on the inflorescences of the giant ginger *C. giganteus*, a regional endemic to the Gulf of Guinea that is widespread in São Tomé between 1200 and 1550 m. Between 1500 and 1700 m, that is, in the *D. santomea* home range, the diversity of alternative resources available and used for feeding by drosophilids is limited and includes mostly the decaying flowers and fruits of *A. vogelii* and the fruits of *C. montanum*. These resources, although suitable to other drosophilids, are not visited by *D. santomea*.