

SHORT COMMUNICATIONS

COMPETITION FOR THE NECTAR OF *CENTROPOGON VALERII* BY THE HUMMINGBIRD *COLIBRI THALASSINUS* AND THE FLOWER-PIERCER *DIGLOSSA PLUMBEA*, AND ITS EVOLUTIONARY IMPLICATIONS

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In the highlands of southern Costa Rica, one of the commonest native plants of second-growth areas is *Centropogon valerii* Standl. (Lobeliaceae), a perennial shrub with orange-red tubular flowers (Wilbur 1972). Although some flowers are produced by *C. valerii* in all months of the year, in the dry season (December through March) a single plant may have over a hundred open flowers on a continual basis. During this flowering peak, most large clumps of *C. valerii* are pollinated primarily by the Green Violet-ear (*Colibri thalassinus cabanidis*) (Trochilidae).

This hummingbird is an altitudinal migrant, spending the rest of the year at lower elevations (Skutch 1967; Slud 1965; see also Wagner 1945; Wolf and Stiles 1970). In the highlands, the male violet-ear often sets up a territory around patches of *C. valerii*, which he defends vigorously against others of his own species, as well as hummingbirds of other species (Colwell 1973; Wolf 1969; Wolf and Stiles 1970). In what must be among the earliest descriptions of territoriality in hummingbirds, Boucard (1878) said of this species: "They take possession of a certain space containing several of these shrubs [very likely *Centropogon*]; and when not feeding on these flowers, they perch on a dry branch near the place, and fight all the other Humming-birds that dare to intrude." Besides other hummingbirds, the Green Violet-ear has two additional potential competitors for the nectar of *C. valerii*, both of them nonpollinating nectar thieves.

The Slaty Flower-piercer (*Diglossa plumbea*) ("Coerebidae") punctures the base of the corolla with its lower mandible and extracts nectar with its tongue, often while perching on the pedicel (in *C.*

valerii), otherwise on a nearby branch (Moller 1931; Skutch 1954; Moynihan 1963). The flower-piercer forages by hopping rapidly from flower to flower, rarely flying more than a few feet. Feeding territories, which often include *C. valerii*, are defended against conspecifics by breeding pairs of flower-piercers during the dry season (see Moynihan 1963, 1968; Skutch 1954; Lyon and Chadek 1971). These territories frequently overlap or include violet-ear territories, so that competition between the two species for the nectar of *C. valerii* is potentially very great.

The second nectar thief is the mite *Rhinoiseius colwelli* Hunter (Mesostigmata; Ascidae) (Hunter 1972), which feeds and breeds inside flowers of *C. valerii*, and is dispersed between flowers on the bill and in the nasal cavities of hummingbirds. A detailed analysis of competitive interactions among three hummingbird species and two *Rhinoiseius* species in the Costa Rican highlands appears elsewhere (Colwell 1973) and includes further data on the species discussed here. The importance of the nectar mite to the present study is its usefulness as an indicator of hummingbird visitation to individual flowers, since nectar mites are not transported by the flower-piercer, nor do they move between flowers on their own power in this plant species. On the basis of crop contents, there is no evidence that either bird species ingests the mites. Likewise, visitation to a flower by the flower-piercer can be inferred by the presence of from one to five punctures in the corolla, between adjacent sepals. Although in more delicate flowers, such as those studied by Lyon and Chadek (1971), both mandibles perforate the corolla, in *C. valerii* only the knife-like lower mandible leaves a puncture, so that the number of punctures in a flower represents the minimum number of visits by flower-piercers to that flower.

Species of the genus *Diglossa* obtain nectar almost exclusively from flowers adapted for hummingbird pollination, so that they characteristically live in sympatry with one or more hummingbird species (see Vuilleumier 1969). The local coexistence of flower-piercers with hummingbirds thus presents an interesting problem, since there is potentially a very high percentage overlap in the nectar resources they depend upon. To the degree that nectar is in short supply in relation to the demand, one would expect the evolution of mechanisms allowing the spatial or temporal partitioning of nectar supplies as a consequence of selection for increased foraging efficiency. These mechanisms might involve both morphological and behavioral traits.

In this study we have analyzed spatial aspects of foraging behavior of the Green Violet-ear and the Slaty Flower-piercer in a relatively simple habitat in which the hummingbird sets up territories comprised exclusively of *Centropogon valerii* shrubs. The violet-ear successfully excludes other hummingbird species from its territories, with the exception of brief visits from Rivoli's Hummingbird (*Eugenes fulgens spectabilis*) at dawn and dusk (Colwell 1973). Though more catholic in its use of nectar sources than the violet-ear, the flower-piercer often concentrates its foraging on *C. valerii* shrubs lying within violet-ear territories.

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To investigate the possibility of resource partitioning on a micro-spatial scale between the two bird species, we first carried out a replicated series of observations on foraging patterns within individual shrubs of *C. valerii*. Every shrub studied was located within the territory of a different male violet-ear, as well as within the territory of a different pair of flower-piercers. After the observational data had been gathered, flowers from specified zones of the very same shrubs were scored for number of nectar mites, as an index of hummingbird visitation, and for number of flower-piercer punctures. These counts allowed an independent check on the observational data.

METHODS

The study area is about 100 m west of the Continental Divide, at 3100 m elevation, near Pension La Georgina, km 88 on the Interamerican Highway, Province of Cartago, Costa Rica (09°34'N; 83°41'W). A detailed description of the site, illustrations of the organisms, and extensive observations in support of those reported here appear elsewhere (Colwell 1973). The field data for the study reported here were taken on 26 March 1971.

Four similar but widely separated large *Centropogon valerii* shrubs were selected in a densely overgrown pasture about 4 ha in area. Each shrub was within the feeding territory of both a violet-ear and a pair of flower-piercers. All four shrubs were observed simultaneously by two-person teams between 07:30 and 10:00. Each team recorded the time of arrival and departure of individual hummingbirds and flower-piercers foraging on the shrub, as well as the position within the shrub of each flower visited. The shrubs were all roughly spherical in outline, 1.5–2.5 m high. For recording the position of flowers, each shrub was considered to have four zones: high-outside, low-outside, high-inside, and low-inside. Any flower below 60 cm above ground level was defined as "low," all others being "high"; any flower within 10 cm of the outer contour of the bush was called "outside," all others being "inside."

At the termination of the observation period, each team selected 10 flowers from each of the four zones on the same bush they had observed, dissected each flower immediately, and scored it for number of nectar mites and number of punctures. Since the male organs mature earlier than the female organs in flowers of *C. valerii*, with each flower producing nectar for 7–12 days (Colwell 1973), it is conceivable that the violet-ear and the flower-piercer feed on the same flowers at different stages of floral development. To test for this possibility, 5 of the 10 flowers from each zone in each shrub were chosen at random from among "young" (staminate stage) flowers, and the other 5 from among "old" (pistillate stage) flowers.

RESULTS

The distribution of visits by the violet-ear and the flower-piercer to individual flowers in the four zones of *Centropogon valerii* shrubs appears in Part IA of table 1. A chi-square test for this 2×4 contingency table strongly rejects ($P < 0.001$) the null hypothesis of homogeneity between the two rows, indicating that the two bird species distribute their foraging activity in different ways among the four zones within *C. valerii* shrubs.

The distribution of nectar mites (indicating previous foraging by the violet-ear) and of punctures (indicating previous foraging by the flower-piercer)

TABLE 1. Frequency of foraging visits, nectar mites, and punctures for flowers of *Centropogon valerii*.

I. Foraging visits, mites and punctures in four zones of <i>C. valerii</i> shrubs.					
A. Numbers of visits by foraging birds ^a					
	Zone ^b				
	OH	OL	IH	IL	
Violet-ear	199	24	34	8	$\chi^2 = 94$
Flower-piercer	62	24	81	28	$P < 0.001$
B. Number of nectar mites and punctures ^c					
	OH OL IH IL				
	OH	OL	IH	IL	
Mites	317	165	237	71	$\chi^2 = 172$
Punctures	23	73	47	94	$P < 0.001$
II. Foraging visits, mites and punctures partitioned by location.					
A. Number of visits by foraging birds ^d					
	O		I		
	O	I	H	L	
Violet-ear	462	56			$\chi^2 = 244$
Flower-piercer	131	207			$P < 0.001$
	H		L		
	H	L	H	L	
Violet-ear	419	99			$\chi^2 = 32$
Flower-piercer	215	123			$P < 0.001$
B. Number of nectar mites and punctures ^e					
	O		I		
	O	I	H	L	
Mites	554	236			$\chi^2 = 126$
Punctures	70	167			$P < 0.001$
	H		L		
	H	L	H	L	
Mites	482	308			$\chi^2 = 31$
Punctures	96	141			$P < 0.001$
C. Number of nectar mites and punctures in flowers in the staminate stage (S, "young" flowers) vs. the pistillate stage (P, "old" flowers). ^f					
	S		P		
	S	P	S	P	
Mites	355	435			$\chi^2 = 1.18$
Punctures	97	140			$P = 0.28$

^a Pooled data from 2.5 hr continuous and simultaneous observation by each of three teams of observers at different shrubs. (The fourth team inadvertently recorded visits only as O or I, and H or L, rather than according to the four zones.)

^b Abbreviations refer to positions of flowers in the shrub: O = Outside, I = Inside, H = High, L = Low.

^c Pooled data from four shrubs. Ten flowers were censused from each of the four zones of each shrub.

^d Pooled data from 2.5 hr continuous and simultaneous observation by each of four teams of observers. Each of the tables in IIA contains all of the data of IA, plus data from the fourth team.

^e Each of the tables in IIB and IIC contains all of the data of IB.

is given in Part IB of table 1. A chi-square test again rejects strongly ($P < 0.001$), indicating a different proportional distribution of mites and punctures, among the four zones of the shrubs.

To discover in what way the observed foraging frequencies differ between the two bird species among zones of *C. valerii*, the data of Part IA of

table 1 were recombined in two ways (outside versus inside, high versus low), yielding the two contingency tables in Part IIA of the table. Chi-square tests for the two 2×2 tables both reject the null hypothesis of independence at $P < 0.001$. From the distribution of visits given in these two 2×2 tables, we infer that the violet-ear forages more frequently on flowers in the outer and upper parts of the shrub, while the flower-piercer, by comparison, concentrates a greater proportion of its foraging activity in the inner and lower parts. In terms of the four zones (Part IA of the table), it is therefore not surprising to note that the ratio of violet-ear to flower-piercer visits is greatest for the outside-high zone and least for the inside-low zone.

Similar treatment of the zone-by-zone data for mites and punctures (Part IB of table 1) supports precisely the same qualitative conclusions. Chi-square tests for the contingency tables of Part IIB of table 1 again reject at $P < 0.001$, for the same reasons, and again the ratio of mites to punctures is highest for the outside-high zone and lowest for the inside-low zone in Part IB of the table.

The striking agreement between the qualitative conclusions from observations of foraging and from censusing of mites and punctures can be seen in figure 1, which presents the data in Part II of the table in terms of percentages.

As a final test of the general conclusion that the violet-ear and the flower-piercer tend to utilize different flowers on *C. valerii* shrubs, a Spearman rank correlation coefficient (corrected for ties) was computed for the number of mites versus the number of punctures in individual flowers, pooling flowers taken from all four zones of the shrubs. The resulting coefficient was highly significant ($P < 0.001$) and negative in sign, indicating as anticipated that flowers with many mites have few punctures and vice versa.

The possibility that the violet-ear and the flower-piercer might be feeding on flowers at different stages of floral development was tested by computing chi-square for the 2×2 contingency table in Part IIC of table 1. As might be expected, "old" (pistillate stage) flowers had more mites and more punctures than "young" (staminate stage) flowers, but there was no significant difference ($P = 0.28$) between the proportional distribution of mites and the proportional distribution of punctures in young and old flowers. We thus infer that either the two bird species do not discriminate between young and old flowers, or that if they do, they discriminate in the same way and to the same degree. Consequently, young and old flowers from each zone of the shrubs were pooled in Part IB of table 1.

It should be emphasized that the number of mites in a flower is simply taken to be an index of hummingbird foraging frequency on that flower. Since the mites reproduce in flowers having an adequate number and sex ratio of adults (see Colwell 1973), and mites may or may not enter or leave a flower at any particular hummingbird visit, this index is not necessarily related in a linear fashion with hummingbird foraging frequency. Nevertheless, number of mites is clearly correlated with foraging frequency, so that tests based only on presence or absence of mites fail to utilize much available information.

Likewise, the number of punctures in a flower is a nonlinear index correlated with the frequency of flower-piercer visits to that flower. The number of pierces represents a minimum estimate of the number of such visits since the same puncture may be used on more than one visit, and once a flower has

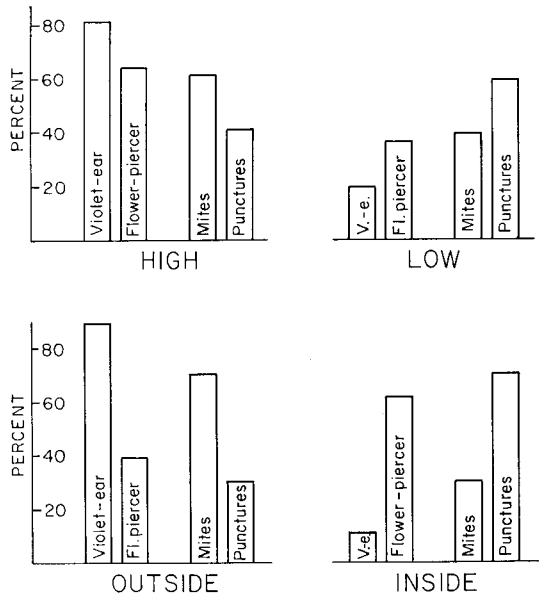


FIGURE 1. Percentage of visits, mites, and punctures partitioned by location. In the upper two histograms, the percentage of foraging visits by each of the two bird species to "high" versus "low" flowers of *Centropogon valerii* is compared to the percentage of mites and punctures in "high" versus "low" flowers. Pairs of corresponding columns of the upper two histograms add to 100%. In the lower two histograms the same kind of comparison is shown for "outside" versus "inside" flowers. Note that in each of the four histograms the pattern of foraging visits is qualitatively very similar to the pattern for mites and piercers. "V-e." = Violet-ear; "Fl.-piercer" = Flower-piercer.

five punctures (one between each pair of adjacent sepals), no more punctures are possible. Again, tests involving mere presence or absence of punctures fail to exploit available information.

Nevertheless, when chi-square tests corresponding to those of Parts IB and IIB of table 1 are performed on tabulation of the number of flowers with one or more mites, versus number of flowers with one or more punctures, the very same qualitative results emerge, with only slightly less statistical significance.

DISCUSSION AND CONCLUSIONS

It is clear that the Green Violet-ear and the Slaty Flower-piercer partitioned the nectar of *Centropogon valerii* on a micro-spatial basis in the shrubs we studied. Without examining foraging patterns of the two species in other habitats, in other parts of their ranges, and in other years and seasons, and without taking into account the non-nectar portions of their diets, we cannot estimate the total degree of potential and actual overlap between their niches. Nevertheless, the data at hand demonstrate at least one ecological difference which seems likely to promote the co-existence of the two bird species.

It might be argued that this pattern is a trivial consequence of intrinsic differences in the functional morphology of hummingbirds and flower-piercers, the former being better equipped for feeding on exposed flowers, and the latter better fitted for foraging on foot in the interior of bushes where perches abound.

In fact, however, each species feeds to some extent on flowers in all zones of *C. valerii* shrubs, including those regions of the plant utilized principally by the other species. Thus, even in the "outside-high" flowers in our samples, 38% of the flowers had punctures made by flower-piercers, while even among the "inside-low" flowers, 53% had mites delivered by the violet-ear. Though their means of approaching flowers to feed are utterly distinct, the agility of the violet-ear on the wing in tight places is matched by the facility of the flower-piercer in using as a perch the pedicel of the very flower it is about to pierce.

We thus infer that the spatial partitioning our data demonstrate is at least in part the product of interference competition. In other words, each of the two species reduces the foraging region of the other (within *C. valerii* shrubs) from what it would be without interference. Although no aggressive interactions between the two species were seen during the study period proper, we have observed such interactions at the study site on numerous other occasions, as well as frequent interactions between the flower-piercer and the Fiery-throated Hummingbird (*Panterpe insignis*). Almost invariably, the flower-piercer escapes by hopping or flying into dense foliage, and the hummingbird gives up the chase (see also Wolf 1969; Moynihan 1963; Borrero 1965; Skutch 1954).

Intending to repeat the study described in this paper, a different group of researchers followed the same protocol at a nearby site in March 1972 (Organization for Tropical Studies 1972). Unfortunately, they failed to ascertain beforehand whether the shrubs chosen were in fact defended by territorial violet-ears. During the observation period, uncontested visits by the Fiery-throated Hummingbird were recorded in the same shrubs (E. Fuentes, pers. comm.) and only 168 flower-visits by violet-ears were observed compared to 518 visits in our study (32% as many). Total flower-visits by flower-piercers were 258, compared to our 338 (76% as many). It is clear that all or most of the four shrubs chosen in the second study were not defended as vigorously by violet-ears as those shrubs observed in our study.

Whatever the reasons for this difference, it provides a convenient test of our hypothesis concerning interference competition. If the intensity of behavioral interference was in fact lower in the later study, we would expect spatial partitioning within shrubs to be less distinct than in our study. The results of the later study confirm this expectation: of the four chi-square tests corresponding to those of table I, Part II, only one was significant at $P < 0.05$ (the outside-inside comparison for foraging visits).

Wolf and Stiles (1970) noted that the outcome of behavioral disputes between the flower-piercer and the Fiery-throated Hummingbird near our study site depended upon the local availability and variety of nectar sources. The hummingbird more consistently displaced the flower-piercer when nectar sources other than the preferred food plant of the hummingbird were readily available. This observation further demonstrates the plasticity of feeding and interference patterns in hummingbird-flower-piercer systems.

Other evidence of interference competition comes from consideration of a second species of *Centropogon* found at the study site, *C. talamancensis* (Wilbur 1969). This plant forms small patches, some of which are interspersed with *C. valerii* shrubs. The flowers are borne only in the axils of the upper leaves on unbranched stalks, so that all flowers are exposed. As

a result of attacks by the violet-ear, *C. talamancensis* flowers within violet-ear territories have significantly fewer flower-piercer punctures than flowers of more isolated *C. talamancensis* plants, undefended by the violet-ear (Colwell 1973), once again indicating displacement of the flower-piercer from a potential source of nectar. The fact that the violet-ear cannot feed on *C. talamancensis* (its bill is too short for the long corolla) only strengthens the case, since it implies that any attack on a trespassing flower-piercer is worth the energetic cost, even with no immediate gain. This could only be the case if the violet-ear benefits to a significant extent from its interference with the flower-piercer on *C. valerii*. It is worth noting that the violet-ear does not attack non-nectarivorous birds in its territory.

From the existence of these feeding and defense patterns, we infer that nectar must be in short supply, at least in some years. If nectar were consistently superabundant, it would be difficult to account for the evolution of such patterns. Thus if either bird species were experimentally excluded from territories of the other, we would expect these territories to contract in size.

It remains to account for the persistence of spatial partitioning in the shrubs we studied, even in the absence of aggressive encounters during the study period proper. Both the flower-piercer and the violet-ear revisit particular shrubs at rather regular intervals, as nectar accumulates in the flowers. Even if the mean interval between visits were the same for the two birds, they would most likely be consistently out of phase as a result of occasional behavioral interactions. Consequently, whenever one of the two species arrives at a shrub, the amount of accumulated nectar per flower will always tend to be lower in the part of the shrub utilized by the other species.

Thus, on shrubs defended by a violet-ear, the flower-piercer forages on low and inside flowers whether or not the violet-ear is actively harassing him at the moment. Individual flower-piercers apparently learn that foraging on exposed flowers of certain shrubs (those exploited and defended by violet-ears) yields little nectar and considerable harassment. Likewise, violet-ears may learn that low and inside flowers on such shrubs have, on the average, less nectar than exposed flowers.

There is another aspect of this system that is of evolutionary interest. Although the Slaty Flower-piercer regularly hawks for flying insects, the bill, tongue, head musculature, and digestive tract are highly modified for nectar feeding, and in many ways parallel similar adaptations in the hummingbirds (Beecher 1951; Moynihan 1963). The flowers it feeds upon are almost exclusively those specialized for pollination by hummingbirds. In both an evolutionary and an ecological sense, then, the flower-piercer is entirely dependent upon the mutualism between hummingbirds and the plants that hummingbirds pollinate.

On the other hand, Lyon and Chadek (1971) argue that the Cinnamon-bellied Flower-piercer (*Diglossa baritula*) has indirectly aided the evolution of ornithophily in flowers of the Guatemalan highlands, since bumblebees tend to extract nectar through *Diglossa* perforations rather than entering flowers through the throat of the corolla, thereby reducing selection for entomophily. However, although bumblebees are common in the Costa Rican highlands, we have never observed this behavior on

flowers of *Centropogon valerii* or any other hummingbird-pollinated plant.

Another interesting evolutionary question is raised by this study. Since the Slaty Flower-piercer frequently concentrates its foraging on the inner and lower flowers of large *C. valerii* plants, but never pollinates the plant, and the violet-ear feeds less frequently on flowers in these parts of the shrub, we might expect selection to favor those plants that tend to produce flowers only in the outer and upper parts. In fact, we found fruit-set to be considerably less consistent in inner and lower flowers, and the density of flowers in these regions is clearly much less than in the outer and upper parts of the shrub.

Two explanations may be offered for the continued presence of flowers in the inner and lower regions of *C. valerii* shrubs in clearings. First, the habit of *C. valerii* in nearby areas undisturbed by human activity is considerably more herbaceous, so that all flowers tend to be low and exposed. These plants are also more sparsely distributed, making their defense difficult and energetically costly. It is not known whether flowers on such plants are differentially utilized by hummingbirds and flower-piercers in undisturbed areas near the study site. It seems likely that they are not, since a feeding flower-piercer is equally vulnerable to hummingbird attack on all flowers, and such attacks are probably less frequent in any case. It may be that selection against interior flower placement in the shrub habit may not yet have taken its course, since large clearings are the result of human activity.

A second and more intriguing possibility is that selection has maintained production of a certain proportion of interior flowers as a means of reducing competition between the "parasitic" flower-piercer and the beneficial hummingbirds. Thus an individual plant may be more likely to become part of a hummingbird feeding territory, and consequently have a higher average rate of seed set per flower, if a reliable supply of nectar can be guaranteed for hummingbirds by providing sufficient interior flowers to divert the activities of flower-piercers. This hypothesis could be tested by removing inner and lower flowers from plants in violet-ear territories and watching for increased interspecific aggression, changes in territorial boundaries in both species, and decreased seed set.

SUMMARY

Competition for the nectar of *Centropogon valerii* (Lobeliaceae) between the Green Violet-ear (*Colibri thalassinus cabanidis*; Trochilidae) and the Slaty Flower-piercer (*Diglossa plumbea*; "Coerebidae") was investigated at a study site in the highlands of Costa Rica. Quantitative observations of foraging behavior were made for individual plants within territories of both bird species. Statistical analysis shows highly significant spatial differences between the foraging patterns of the two species, the hummingbird concentrating on flowers in the outer and upper parts of the shrub, while the flower-piercer feeds relatively more frequently on inner and lower flowers.

The presence of nectar mites *Rhinoseius colwelli* in an individual flower indicates previous hummingbird visits, while the presence of punctures in the corolla is evidence of previous foraging by the flower-piercer. The qualitative conclusions on foraging pattern obtained from observational data are exactly paralleled by conclusions from census data for nectar

mites and punctures in flowers taken from different spatial zones of the same shrub. Furthermore, number of nectar mites is negatively correlated with number of punctures for all flowers pooled.

The evidence suggests that the observed spatial partitioning of flowers within *C. valerii* shrubs is the result of interference competition based on behavioral interactions and energetic factors.

The flower-piercer is dependent in both an evolutionary and an ecological sense on the mutualism between hummingbirds and hummingbird-pollinated plants. Since inner and lower flowers of *C. valerii* are infrequently pollinated, it may be that they serve to divert flower-piercers from flowers more accessible to hummingbirds, thus increasing the probability of inclusion of the plant in a violet-ear territory, and increasing total seed set.

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RELATIONSHIP OF GONADAL RECRUDESCENCE AND TESTICULAR MELANOGENESIS IN CALIFORNIA QUAIL

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The testes, but usually not the ovary, of many unrelated species of birds undergo considerable seasonal variation in color. Testes of these birds are generally pearl-white during the breeding season, but during testicular regression and postnuptial molt, they change in color, becoming dark to black. In other species of birds the testes may be yellow-orange in coloration (Serventy and Marshall 1956). The California Quail (*Lophortyx californicus*) in southeastern Washington exhibits a seasonal change in testis coloration. During fall and winter the testes of these quail are dark blue-black, but with the onset of recrudescence in the spring, they become lighter in color. At the height of breeding condition, the testes are pearl-white, and with testicular regression during late summer, the testes return to the blue-black color. This color change, which occurs with testicular recrudescence and regression, appears to be associated with the amount of melanin pigment in the interstitial tissue of the testis. Serventy and Marshall (1956) stated that this color change is the result of pigment dispersal with increase in testicular volume during recrudescence. According to J. King, Washington State University (pers. comm.), black testes seem to be unusual in birds, occurring in only a small proportion of specimens from a given species and locality. Studies on the gonadal cycle of *Lophortyx* spp. (Jones 1970; Williams 1967; Raitt and Ohmart 1966) do not mention a seasonal change in testes color; however, Lewin (1963) qualitatively described the abundance of pigment in microscopic sections of recrudescing and regressing testes of California Quail. The first author of this paper observed black testes in Gambel's Quail (*Lophortyx gambelii*) in southern Arizona and in Bobwhite (*Colinus virginianus*) in north-central Kansas during the fall months.

The reproductive biology of quail of the genus *Lophortyx* has previously been described (Jones 1970; Anthony 1970; Williams 1967; Raitt and Ohmart 1966; Lewin 1963). These studies outline the seasonal spermatogenic cycle or changes in interstitial cell activity of California Quail and Gambel's Quail.

Fletcher (1971) studied the effect of vitamin A deficiency on the pituitary-gonadal axis and reproductive performance in California Quail. The purpose of this paper is to show that testis coloration is controlled, in part, by the interstitial melanophore, whose function is related to breeding condition in California Quail. The above papers have not described this relationship, even though they mention the abundance of melanin pigment in the interstitium of the testis. We will also suggest the effect of the hormones of reproduction on melanogenesis and the functioning of the "interstitial melanin unit."

MATERIALS AND METHODS

California Quail were collected in the field by shooting from February until October 1967. Testes were removed immediately after collection and placed in AFA solution. They were allowed to remain in this fixative for approximately 24 hr, at which time they were transferred to a 10% formalin solution for storage.

Testes were weighed (from formalin) to the nearest 0.01 g, and the length and width were measured to the nearest 0.1 mm. The volume of each testis was calculated using the formula for the volume of an ellipsoid: $V = \frac{4}{3} \pi ab^2$, where a is $\frac{1}{2}$ the length and b is $\frac{1}{2}$ the width. Microslides were prepared routinely of the left testis from each quail collected. Tissues were dehydrated through the standard alcohol-benzene series and embedded in Waterman's paraffin. Sections were taken from approximately the middle of each testis, cut at 8 μ , and stained with Heidenhain's hematoxylin.

Aggregated lipids were washed out by the alcohol-benzene series, so they would not appear in sections of testicular tissue. This treatment facilitated the analysis of melanin pigment in the interstitium.

The technique employed by Krumrey and Buss (1969) was used to analyze each testis slide. A reticule with a 200-square grid was inserted into the drawtube of a microscope. The reference points used in the cell counts were the 200 intersection points of the squares. The number of intersections which fell on melanin granules was counted and expressed as a percentage of the total number of intersections. These data were used as a relative indication of the amount of melanin in the interstitium. To eliminate bias, fields within slides were selected by the use of the table of random numbers obtained from Fisher and Yates (1949:104-109). Sample size was determined by Stein's formula (Steel and Torrie 1960:86).

RESULTS

Spermatogenesis. The histological sequence of recrudescence and regression in California Quail has been described by Lewin (1963) and quantified by Anthony

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