WING-SCALE PATTERN VARIATION IN *ANOPHELES PUNCTIPENNIS* (SAY)

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ABSTRACT. The presence and relative size of the subcostal pale spot to the preapical dark spot (SCP ratio) is thought to be an important diagnostic character for identifying adult *Anopheles punctipennis*. Variability of the SCP ratio on the wings of *An. punctipennis* was determined for four populations from California and Illinois. The SCP ratio was highly variable between individuals collected in the field and some individuals lacked the SCP altogether. SCP ratios were equally variable among siblings from isofemale lines. The SCP ratio, alone, is not a useful taxonomic character for distinguishing *An. punctipennis* from the closely related species, *An. perplexens*. Variability in the extent or presence of pale scaling on other wing veins suggests that patterns on individual veins are not particularly useful as diagnostic taxonomic characters.

INTRODUCTION

The pattern and color of scales on the wings of anopheline mosquitoes are important characters for distinguishing species and higher taxonomic groups (Wilkerson and Peyton 1990). As with many anatomical characters, however, much intraspecific variation may be encountered when numerous specimens are sampled throughout a species' range (McClelland 1974). Thorough study of character variability within and between species is thus important in order to avoid overly simplistic identification keys. Such variation is normally not a problem when combinations of characters are used to distinguish species. Problematic identifications occur when only one or a few variable characters exist to distinguish closely related species.

During an investigation into the population biology of *Anopheles punctipennis* (Say) at Lake Vera in Nevada County, California, we observed substantial variation in wing-scale patterns. In several instances, the specific identity of some individuals was inconclusive, since they lacked wing-scale patterning associated with this species. Many individuals matched the description of a closely related species, *An. perplexens* Ludlow (Darsie and Ward 1981).

Anopheles perplexens was described by Ludlow (1907) from a single specimen collected in Pennsylvania. What distinguished this specimen from *An. punctipennis* was the reduction of pale-scaled areas throughout the wings. Darsie and Ward (1981) separate both species on the basis of the ratio of the length of the subcostal pale spot (SCP) to the preapical dark spot (PD) (nomenclature of Harbach and Knight 1980, Wilkerson and Peyton 1990). The ratio for *An. punctipennis* is 0.5 or more and the ratio for *An. perplexens* is usually 0.33 or less. Although Howard et al. (1917) considered *An. perplexens* an aberrant melanistic form of *An. punctipennis*, Bellamy (1956) concluded that *An. perplexens* was a good species based on a variety of anatomical, ecological, biological, and behavioral observations. Bellamy (1956) stated that the most reliable means of distinguishing both species was by egg morphology. He also noted that adults of *An. perplexens* had substantially reduced subcostal pale spots on the wings. Kreutzer and Kitzmiller (1971) studied the polytene chromosomes of both species and concluded that, overall, the differences were slight and hardly conclusive in judging their validity as separate species. The X chromosome, however, showed the most difference and supported the separation of both species. Although Kreutzer and Kitzmiller (1971) stated that the X chromosome may be reliably used to distinguish *An. perplexens* from *An. punctipen-*

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nis, Fritz et al. (1991) have shown that conclusions about the diagnostic value of minor chromosomal differences are premature until intraspecific variability has been determined for both species throughout their range.

Bellamy (1956) found no diagnostic characters for separating individual larvae of *An. punctipennis* from those of *An. perplexens*. However, the majority of 4th instar larvae of *An. punctipennis* had seta 2 on abdominal segments IV and V split into two or more branches; seta 2 of *An. perplexens* was usually single.

At present there are no records of *An. perplexens* west of Ohio. This species is known only from regions along the Gulf and Atlantic coasts as far north as New York, and in restricted inland areas of Georgia, Alabama, Florida, Pennsylvania, Tennessee, Ohio and North Carolina (Darsie and Ward 1981). Alternatively, *An. punctipennis* is found throughout the continental U.S. except for Alaska, Nevada, Utah and Arizona.

The purpose of this paper is not to question the validity of *An. perplexens* as a separate species. We concur with Bellamy (1956) that *An. perplexens* is a separate species, based on his extensive and convincing comparisons with *An. punctipennis*. Rather, we address the usefulness of a particular character, the SCP ratio (Darsie and Ward 1981), that has been used to distinguish both species. Since cryptic species are common in the genus *Anopheles*, the possibility arose that two or more species were being represented in our study on the biology of *An. punctipennis* in California. The objective of this investigation, therefore, was to determine whether the SCP ratio was a useful character for indicating the presence of *An. perplexens*, or other cryptic species, in collections of *An. punctipennis* from the field.

MATERIALS AND METHODS

Larvae of *An. punctipennis* were collected from three geographically distant areas of California and reared to adults: Willits in Mendocino Co., Woodlake in Tulare Co. and Lake Vera in Nevada Co. Some larvae from the Lake Vera site were reared in individual vials in order to obtain larval and pupal exuviae. A sample of the larval exuviae from specimens that fit the descriptions (SCP ratio) of *An. perplexens* and *An. punctipennis* were analyzed for the condition of seta 2 on abdominal segments IV and V. Adult mosquitoes were also collected from an area in an around Allerton Park near Monticello, Illionois. This site was the same location where Baker and Kitzmiller (1964) collected specimens for their description and map of the polytene chromosomes of *An. punctipennis*. Only the left wings of a random sample of females from each collection site were used in the analysis of wing-scale pattern variation. Measurements of the ratio of the subcostal pale spot (SCP) to the preapical dark spot (PD)(SCP ratio) were made with a dissection microscope fitted with an ocular micrometer. To assess intraspecific variation, SCP ratios were also determined for individuals from single family lines obtained from females collected in Illinois. Eggs obtained from all females were compared to the descriptions of eggs from *An. perplexens* (Bellamy 1956) and *An. punctipennis* (Herms and Freeborn 1920, Herms and Frost 1932, Aitken 1945, Bellamy 1956).

Since the range of variation in wing-scale pattern appeared to be greatest in individuals collected from the site at Lake Vera, California, an enzyme electrophoretic analysis was done in order to identify any population substructuring or cryptic species at this location. Adult females were collected during several consecutive nights and electrophoresed on horizontal starch gel in the manner described by Steiner and Joslyn (1979). A chi-square analysis of polymorphic enzyme loci was done to determine the fit of genotype frequencies to Hardy-Weinburg equilibrium expectations.

RESULTS

A large amount of variability in the SCP ratio was observed in all four samples of field collected *An. punctipennis* (Fig. 1). Most individuals had SCP ratios that were intermediate between those ratios stated by Darsie and Ward (1981) as specific to *An. punctipennis* and *An. perplexens*. From 12-23% of the SCP ratios for individuals collected in the field corresponded to those expected for *An. perplexens*. The SCP ratios

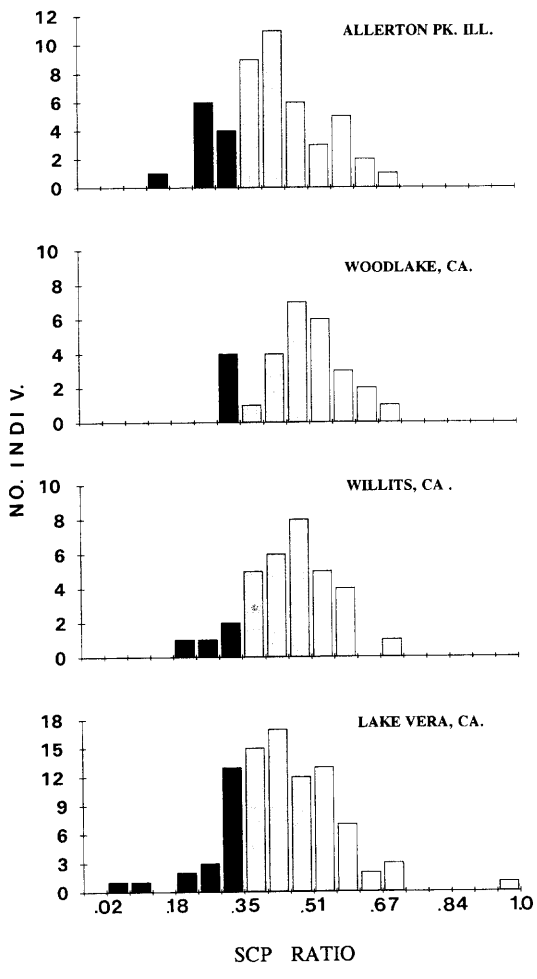

Fig. 1. SCP ratios of *An. punctipennis* collected from four sites. Black columns are ratios in the range of *An. perplexens* and clear columns are those in the range of *An. punctipennis* (Darsie and Ward 1981).

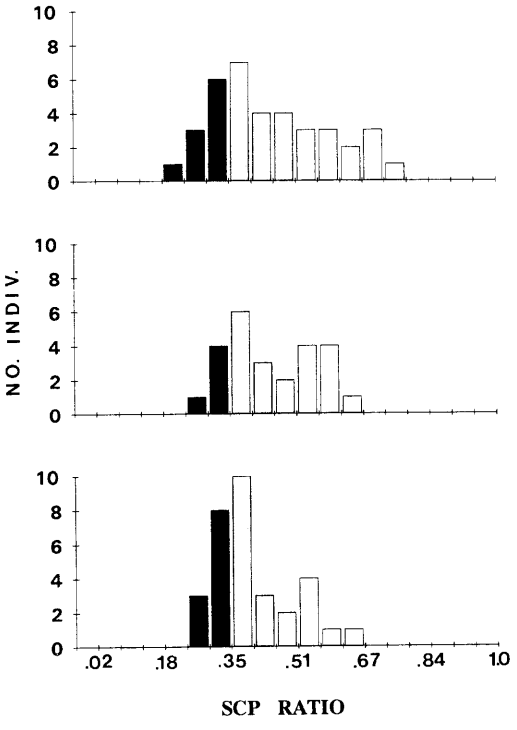

Fig. 2. SCP ratios for progeny from three separate isofemale lines of *An. punctipennis*. Black columns are those ratios in the range of *An. perplexens* and clear columns are those in the range of *An. punctipennis* (Darsie and Ward 1981).

obtained from single family progeny were equally variable as those obtained from individuals collected in the field (Fig. 2).

The number of branches of seta 2 on abdominal segments IV and V of larval exuviae originating from females with SCP ratios in the range of *An. perplexens* were not different from those obtained from individuals with SCP ratios in the range of *An. punctipennis* (Table 1).

We obtained eggs from 25 females collected in Illinois and found no batch that corresponded to the type described for *An. perplexens* by Bellamy (1956). Over the past 1½ years we have also collected *An. punctipennis* from various locations throughout California and have not seen the eggs of *An. perplexens* represented in our samples.

Eight polymorphic loci were found in the electrophoretic analysis of *An. punctipennis*: aconitase (Acon-1), phosphoglucomutase (Pgm-1), isocitrate dehydrogenase (Idh-1, Idh-2), glutamate oxaloacetate transaminase (Got-1, Got-2), hydroxyacid dehydrogenase (Had-1) and phosphoglucose isomerase (Pgi-1). There were no significant differences in the frequencies of observed genotypes from those expected under Hardy-Weinberg equilibrium (Table 2).

DISCUSSION

The SCP ratios of all field collected samples indicate that the subcostal pale spot is a highly variable character. Either mosquitoes collected at each site actually represented two or more species, or SCP ratio variation is simply an in-

traspecific variable character. If one uses the criterion that *An. punctipennis* has a SCP ratio of 0.5 or more (Darsie and Ward 1981), then most individuals in our samples would have questionable species designation. The measurements of SCP ratios for progeny from single families, however, demonstrates that the large amount of variability observed in field populations can be accounted for by intraspecific variation. There was also no evidence from the examination of eggs or larval chaetotaxy that supported the hypothesis of two or more species. Neither is there mention in various descriptions of *An. punctipennis* eggs in California (Herms and Freeborn 1920, Herms and Frost 1932, Aitken 1945) of eggs that correspond to those described for *An. perplexens.*

Further evidence of intraspecific variation of the SCP ratio is provided by the electrophoretic analysis of individuals collected at Lake Vera. Although the range of SCP values was greatest at this site, the expected frequencies of genotypes at all eight polymorphic loci were not significantly different from those expected for a single randomly mating species (absence of the Wahlund effect). It would be extremely unlikely that cryptic species were present there that shared the same electromorphs at identical frequencies for eight separate enzyme loci. If a cryptic species were present at Lake Vera, then its frequency must be insignificant for the purpose of this investigation.

Although this paper focuses on the subcostal pale spot, all other pale-scaled areas of the wings were cursorily examined. In some specimens collected at Lake Vera, pale scales were completely absent on the costa and R_1 veins. The amount of pale scaling on other specimens was so minimal that these individuals were initially identified in the field as *An. freeborni.* We concur with Ross (1947) who stated that, "Two wing spots appear to be constant, the apical costal spot and the white bar on the base of the anal vein. All other spots vary greatly, and occasionally additional white bars appear on practically all the radial and medial veins. The preapical spot (SCP) is subject to the most conspicuous variation."

From the time *An. perplexens* was described by Ludlow (1907), and Bellamy (1956) had convinced most culicidologists that this species was valid, references to dark forms of *An. punctipennis* have been referred to as "*perplexens*" or "*perplexens*-like". The implicit assumption for collection records, where both dark and light forms are mentioned, has been that both species were present at these sites. It is clear from this study that the SCP ratio in *An. punctipennis* is highly variable and should not be used exclusively to distinguish this species from others. Some individuals lack this spot altogether, which was also observed by Ross (1947). Keys to the anopheline mosquitoes of North America invariably refer to the SCP spot or other pale scale spot on the wings of adults as important characters for identifying *An. punctipennis* (Aitken 1945, Matheson 1929, Freeborn 1949, Vargas and Martinez Palacios 1956, Darsie and Ward 1981, Wilkerson and Strickman 1990). Although the overall pattern of white scales is shared by

Table 1. The number of branches for seta 2 on the left and right sides of abdominal segments IV and V of fourth-instar larval exuviae from adults with SCP ratios in the range of *An. perplexens* and *An. punctipennis.*

	Seg IV		Seg. V			Seg. IV		Seg. V	
SCP ratio	L	R	L	R	SCP ratio	L	R	L	R
0.33	2	2	2	2	0.52	2	2	2	--
0.04	2	2	2	2	0.57	3	3	--	3
0.21	3	2	3	3	0.53	3	3	3	4
0.35	2	2	--	2	0.48	1	--	2	2
0.39	2	--	2	2	0.64	1	--	--	2

Table 2. Observed and expected numbers of genotypes for eight polymorphic enzyme loci in *An. punctipennis* collected at Lake Vera, California.

	OBS.	EXP.
ACON-1		
AA	55	55.58
AB	5	4.56
AC	8	7.29
BB	0	0.07
BC	0	0.30
CC	0	0.21
CHI-SQ = 0.70		
P > 0.87		
GOT-1		
AA	61	61.00
AB	1	1.00
BB	0	0
CHI-SQ = 0		
P = 1.00		
GOT-2		
AA	56	56.12
AB	5	4.80
AC	1	1.00
BB	0	0.08
BC	0	0.04
CC	0	< 0.01
CHI-SQ = 0.13		
P > 0.98		
IDH-1		
AA	41	40.23
AB	11	12.89
AC	1	0.86
AD	1	0.86
BB	2	0.95
BC	0	0.14
BD	0	<0.01
CD	0	0.01
DD	0	<0.01
CHI-SQ = 1.78		
P > 0.93		
HAD-1		
AA	11	12.13
AB	26	24.75
AC	2	0.99
BB	12	12.13
BC	0	0.99
CC	0	0.01
CHI-SQ = 2.19		
P > 0.53		
PGI-1		
AA	27	27.02
AB	11	10.97
BB	1	1.02
CHI-SQ < 0.01		
P > 0.98		
PGM-1		
AA	32	32.85
AB	4	3.65
AC	4	3.65
BB	0	0.08
BC	0	0.20
CC	0	0.08
CHI-SQ = 0.43		
P > 0.98		
IDH-2		
AA	52	52.00
AB	5	4.83
BB	0	0.09
CHI-SQ = 0.95		
P > 0.75		

most individuals in our collections, single patches of white scales on most of the wing veins vary in number and size. It is clear from this study that the SCP ratio alone is not useful for distinguishing *An. perplexens* from *An. punctipennis*. At present, the only character that may be useful in distinguishing individuals of both species is egg anatomy.

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