

Comparative study on the relationship between photoperiodic host-seeking behavioral patterns and the eye parameters of mosquitoes

Hitoshi Kawada^{a,*}, Haruki Tatsuta^b, Kentaro Arikawa^c, Masahiro Takagi^a

^aDepartment of Vector Ecology & Environment, Institute of Tropical Medicine, Nagasaki University, Nagasaki, Nagasaki 852-8523, Japan

^bLaboratory of Ecological Risk Assessment, National Institute for Environmental Studies, Tsukuba, Ibaraki 305-8506, Japan

^cLaboratory of Neuroethology, Graduate School of Integrated Science, Yokohama City University, Yokohama, Kanagawa 236-0027, Japan

Received 23 May 2005; accepted 20 September 2005

Abstract

Relationships between the ommatidial structure and photoperiodic behavior of several mosquito species were investigated. Host-seeking behavioral patterns of mosquitoes were classified into four main groups based on previously compiled reports on field or laboratory biting activity. These groups were pattern I and I' (nocturnal), pattern II (crepuscular and nocturnal), pattern III (crepuscular and diurnal), and pattern IV (diurnal). Eye parameters (product of facet diameter and interommatidial angle) of mosquitoes that belong to the pattern I and I' group were higher (2.7–4.2) than those of mosquitoes that belong to the pattern IV group (0.8–2.3). Eye parameters of the mosquitoes categorized in the pattern II and III groups were intermediate (2.3–2.6). These results suggest that the crepuscular behavior of mosquitoes undergoes a transition in the course of evolution from nocturnal behavior to diurnal behavior. Large variations in the eye parameters were observed even within the same genus depending on their photoperiodic behavior. Therefore, the ommatidial structure of mosquitoes appears to be determined, not taxonomically, but evolutionarily by the photoenvironment in which the mosquitoes are most active.

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Keywords: Eye parameter; Mosquito; Host-seeking pattern; Behavior; Ommatidia

1. Introduction

Mosquitoes cover a wide range of light environments associated during their performance of various activities such as host seeking, blood feeding, mating, and oviposition. The family Culicidae includes mosquitoes with various lifestyles ranging from the completely nocturnal *Anopheles* spp. to the diurnal *Toxorhynchites* spp. Most mosquitoes have apposition eyes and are active at either dusk or night. Generally, in nocturnal mosquitoes, the lens diameter is relatively large as compared to the depth of the dioptric apparatus, and the rhabdoms are short, fused, and conical. On the other hand, in diurnal mosquitoes, the aperture of the lens is small and the rhabdomeres are longer than those in the nocturnal species (Land et al., 1999). Land et al. (1997) reported that the entirely

nocturnal *Anopheles gambiae* has apposition eyes that consist of short and thick rhabdoms and interommatidial angles ($\sim 8^\circ$) that are larger than those in other dipteran insects. On the other hand, the diurnal *Toxorhynchites brevipalpis* has neural superposition eyes with lenses that have a small diameter and small interommatidial angles ($\sim 3^\circ$). Land et al. (1999) attempted to establish the entire range of ommatidial morphologies across Culicidae by analyzing the morphological data for six species reported by Sato (1953a,b, 1957, 1959, 1960, 1961) and Sato et al. (1957) and those for six other newly examined species. They suggested that the possible arrangement in mosquitoes from classical apposition eyes to neural superposition eyes may be a missing link in the evolutionary scheme of the Diptera. Although there was no evolutionary or taxonomical substantiation, based on the hypothesis that the neural superposition eyes could be derived from the apposition eyes, Land et al. (1999) placed crepuscular mosquitoes in the intermediate group, i.e., between the

*Corresponding author. Tel.: +81 958 497 811; fax: +81 958 497 812.
E-mail address: vergiss@net.nagasaki-u.ac.jp (H. Kawada).

nocturnal and diurnal mosquitoes (Nilsson and Ro, 1994; Melzer et al., 1997). The existence of species that have intermediate features in the eye system, such as *Armigeres subalbatus* and *Sabethes cyaneus*, might provide evidence for the above hypothesis.

Eye parameter (EP)—a product of facet diameter (D , μm) and interommatidial angle ($\Delta\phi$, radian)—provides a measure of the sensitivity of insect eyes. Most insects operating predominantly in bright light have an EP value of $0.45 \leq \text{EP} \leq 1$ and those of crepuscular insects and nocturnal insects are $1 \leq \text{EP} \leq 2$ and $2 \leq \text{EP} \leq 3$, respectively. Thus, the dimmer the insect's environment, the larger is its EP (Snyder, 1979). A few reports are available on the EPs of mosquitoes; EP = 3.8 for *An. gambiae* (Land et al., 1997; Clements, 1999), and EP = 1.1 for *Tx. brevipalpis* (Clements, 1999). Based on the data of *Aedes aegypti* by Muir et al. (1992), Clements (1999) calculated the EP to be 1.9. Recently, Kawada et al. (2005) reported a significant difference in the ommatidial diameters, interommatidial angles, and EPs between *Ae. aegypti* (EP = 2.1) and *Ae. albopictus* (EP = 1.6). These differences indicated that *Ae. albopictus* adapts to or prefers a brighter environment than *Ae. aegypti*. Based on the categorization by Snyder (1979), the EPs for the four mosquito species mentioned above seem to explain the mutual adaptation of EPs and their behavioral pattern. However, the data required to discuss the entire range of ommatidial morphologies across Culicidae is insufficient. Additionally, it is necessary to focus on the species that exhibit photoperiodic behavior that is intermediate between nocturnal and diurnal. We should also focus on the species that show “exceptional” photoperiodic behavior among taxonomically congeneric species. In this study, we investigated the relationship between the ommatidial structure represented by EP and the photoperiodic host-seeking behavior of several mosquitoes. We also attempted to establish a theory that the sequential evolution of photoperiodic host-seeking pattern of mos-

quitoes is closely related to the structural evolution of mosquito eyes.

2. Materials and methods

2.1. Mosquito species examined

Laboratory colonies or wild *Culex quinquefasciatus* (Say), *Cx. pipiens pallens* (Coquillett), *Cx. pipiens molestus* (Forsk.) (Forskal), *Cx. tritaeniorhynchus* (Giles), *Anopheles dirus* (Peyton and Harrison), *An. minimus* (Theobald), *An. balabacensis* (Baisas), *An. albimanus* (Wiedemann), *An. stephensi* (Liston), *An. soperi* (Bohart and Ingram), *Aedes aegypti* (L.), *Ae. albopictus* (Skuse), *Ochlerotatus togoi* (Theobald), *Oc. japonicus* (Theobald), *Oc. taeniorhynchus* (Wiedemann), *Armigeres subalbatus* (Coquillett), and *Trirap- teroides bambusa* (Yamada) were used. The site of collection, collection date, and institution of source are listed in Table 1. The mosquitoes were maintained in a laboratory after collection or after introduction at 27 °C, 70% RH, and a 16:8 LD-photoperiod regime.

2.2. Measurement of eye parameter

Female mosquitoes were killed by chloroform, decapitated, and prefixed in 2% paraformaldehyde and 2% glutaraldehyde in 0.1 M sodium cacodylate trihydrate buffer (pH 7.3) (CB) for 2 h. Following prefixation, the samples were rinsed in 0.1 M CB for 30 min (three times) and postfixed with 2% osmium oxide in 0.1 M CB for 1.8 h. The samples were then rinsed in 0.1 M CB and dehydrated in a graded series of ethanol and acetone and finally rinsed with dehydrated acetone for 20 min (twice). Acetone was replaced with propylene oxide, and the samples were immersed in a resin solution (epoxy resin + propylene oxide) for 24 h. Following immersion, propylene oxide was evaporated in a drying box with desiccants (silica gel) for one or two nights. The samples were embedded in

Table 1
Mosquitoes used in the study

Species	Site of collection	Collection date	Institution of source
<i>An. dirus</i>	Thailand	February, 2000	Nagasaki University, Japan
<i>An. balabacensis</i>	Lombok, Indonesia	October, 2004	Wild colony
<i>An. minimus</i>	Chaingmai, Thailand	1995	Nagasaki University, Japan
<i>An. stephensi</i>	India	1950s	Nagasaki University, Japan
<i>An. albimanus</i>	—	—	London University, GB
<i>An. soperi</i>	Okinawa, Japan	January, 2005	Wild colony
<i>Cx. quinquefasciatus</i>	Malaysia	—	Universiti Sains Malaysia
<i>Cx. pipiens pallens</i>	Tokyo, Japan	2004	National Institute of Infectious Diseases, Japan
<i>Cx. pipiens molestus</i>	Tokyo, Japan	2004	National Institute of Infectious Diseases, Japan
<i>Cx. tritaeniorhynchus</i>	Saga, Japan	1998	Nagasaki University, Japan
<i>Oc. togoi</i>	Nagasaki, Japan	December, 2004	Wild colony
<i>Oc. japonicus</i>	Nagasaki, Japan	December, 2004	Wild colony
<i>Oc. taeniorhynchus</i>	USA	—	University of Florida, USA
<i>Ar. subalbatus</i>	Okinawa, Japan	November, 2004	Wild colony
<i>Tr. bambusa</i>	Saga, Japan	December, 2004	Wild colony

epoxy resin and stored in a drying oven at an initial temperature of 37 °C for 2 h and 60 °C for the following 48 h. The samples were cut vertically to the line which connects the base of proboscis to the cervix. Sections with a thickness of 1 µm were cut with an ultra microtome and stained with a mixed solution of toluidine blue and azur II (Wako Pure Chemical Industries, Ltd., Osaka, Japan). Digital photographs of the sections were taken using a light microscope. The facet diameter and interommatidial angle, i.e., the angle between the axes of adjacent ommatidia, were measured based on the photographs of the ommatidial longitudinal sections of the lateral or the ventral parts of the compound eye. The ommatidial axis was considered as a line passing through the midpoint of the rhabdom and the corneal lens. The interommatidial angle was measured from the line drawings. Four to six adjacent ommatidia were selected per sample and the mean ommatidial diameter (D , µm) and the mean interommatidial angle

($\Delta\phi$) were calculated. The EP was calculated as a product of D and $\Delta\phi$ (converted in radian).

2.3. Data analysis

Optical characteristics of mosquitoes, such as facet diameters, interommatidial angles, and EPs, were compared using one-way analysis of variance (ANOVA) and multivariate analysis of variance (MANOVA), the latter is used for comparing multiple characters simultaneously. In order to examine the optical characteristics that are significantly related to host-seeking behavioral patterns, canonical variate analysis (CVA) (Marcus, 1990) was performed. CVA is particularly useful for finding a quantitative classification rule (called linear discriminant function) using the values of explanatory variables to predict membership of an object in one of the prespecified classes (Lepš and Šmilauer, 2003). In CVA, coefficients of

Table 2
Optical characteristics of mosquito eyes and host-seeking pattern

Species	Facet diameter (µm) (95% CL) ^c	Interommatidial angle (deg) (95% CL)	Eye parameter (95% CL)	Photoperiodic seeking pat	Reference
<i>An. gambiae</i> ^a	28.0	6–10	3.8	I	Pates and Curtis (2005)
<i>An. dirus</i>	22.1 (21.3–23.0)	8.1 (6.7–9.5)	3.1 (2.5–3.9)	I, I'	Pates and Curtis (2005) Dutta et al. (1996) Schultz (1992)
<i>An. balabacensis</i>	20.3 (18.9–21.8)	7.6 (6.8–8.4)	2.7 (2.5–2.9)	I, I'	Scanlon and Sandhinand (1965)
<i>An. minimus</i>	20.0 (19.3–20.8)	8.1 (7.5–8.7)	2.8 (2.7–2.9)	I, I'	Pates and Curtis (2005) Chareonviriyaphap et al. (2003)
<i>An. stephensi</i>	25.7 (24.5–26.8)	7.7 (7.3–8.1)	3.4 (3.1–3.8)	I	Pates and Curtis (2005) Kawada and Takagi (2004) Manoucheri et al. (1976)
<i>An. albimanus</i>	29.3 (24.0–34.6)	8.2 (6.2–10.2)	4.2 (3.9–4.4)	I	Hobbs et al. (1986)
<i>An. soperi</i>	20.0 (19.0–21.0)	4.1 (3.6–4.7)	1.4 (1.2–1.7)	IV	Toma and Miyagi (1986) Kawada and Takagi (2004)
<i>Cx. quinquefasciatus</i>	26.9 (25.1–28.7)	6.5 (6.1–6.9)	3.0 (2.7–3.4)	I	Pipitgool et al. (1998) Sucharit et al. (1981)
<i>Cx. pipiens pallens</i>	23.1 (20.3–25.9)	6.9 (5.9–7.8)	2.8 (2.2–3.4)	I	Omori and Fujii (1953)
<i>Cx. pipiens molestus</i>	28.0 (27.6–28.5)	5.2 (4.0–6.4)	2.6 (2.0–3.1)	II	Chiba et al. (1982)
<i>Cx. tritaeniorhynchus</i>	27.6 (25.8–29.3)	5.4 (4.6–6.2)	2.6 (2.2–2.9)	II	Kawada and Takagi (2004) Sonoda (1971)
<i>Ae. aegypti</i> ^a	17.2	6.2	1.9	IV	Kawada and Takagi (2004) Chadee and Martinez (2000)
<i>Ae. aegypti</i> ^b	18.7 (18.1–19.3)	6.4 (6.3–6.5)	2.1 (2.0–2.2)	IV	Trpis et al. (1973)
<i>Ae. albopictus</i> ^b	17.1 (16.3–17.9)	5.3 (5.1–5.5)	1.6 (1.5–1.7)	IV	Kawada and Takagi (2004) Ho et al. (1973)
<i>Oc. togoi</i>	26.5 (24.0–28.9)	6.5 (6.1–6.9)	3.0 (2.7–3.3)	I'	Omori and Fujii (1953)
<i>Oc. japonicus</i>	24.3 (22.2–26.3)	5.5 (4.5–6.5)	2.3 (2.1–2.5)	III	Chiba (1971)
<i>Oc. taeniorhynchus</i>	25.4 (23.5–27.4)	5.1 (4.8–5.3)	2.3 (2.0–2.5)	II	Nayar and Sauerman (1971)
<i>Ar. subalbatus</i>	22.6 (20.5–24.7)	5.8	2.3 (2.1–2.5)	III	Pandian (1994) Chiba (1971)
<i>Tr. bambusa</i>	16.4 (14.6–18.2)	0.96 (0.72–1.2)	0.83 (0.62–1.04)	IV	Miyagi (1973)
<i>Tx. brevipalpis</i> ^a	24.9	2.6	1.1	IV	Clements (1999)

^aClements (1999).

^bKawada and Takagi (2004).

^c95% confidence limit.

explanatory variables are calculated in order to obtain the largest among-group variance to within-group variance ratio. Ninety-five percent confidence ellipses of the centroid of the first two canonical variate scores were calculated for each host-seeking pattern. Linear discriminant function was used to examine the number of individuals correctly classified into the original group of host-seeking patterns and of taxonomic status.

3. Results

3.1. Classification of photoperiodic host-seeking pattern

To understand differences in host-seeking behavior, we classified the typical behavioral patterns of host-seeking mosquitoes into four main categories based on previous reports from the field or laboratory (Table 2). The four groups were as follows: pattern I and I' (nocturnal), pattern II (crepuscular and nocturnal), pattern III (crepuscular and diurnal), and pattern IV (diurnal). Behavioral patterns I', II, and III may include several subpatterns composed of several peaks with different magnitudes (Fig. 1). *An. dirus*, *An. balabacensis*, *An. minimus*, *An. stephensi*, *An. albimanus*, *Cx. quinquefasciatus*, *Cx. pipiens pallens*, and *Oc. togoi* are classified into the group having the pattern I or I' group. Among these, *An. stephensi*, *An. albimanus*, *Cx. quinquefasciatus*, and *Cx. pipiens pallens* are reported to have clear unimodal nocturnal host-seeking patterns, while the others show bimodal patterns or a higher number of peaks. *Cx. pipiens molestus*, *Cx. tritaeniorhynchus*, and *Oc. taeniorhynchus* are classified into the pattern II group, in

which both crepuscular and nocturnal biting peaks are found. *Oc. japonicus* and *Ar. subalbatus* are classified into the pattern III group. *An. saporoi* and *Tr. bambusa* have clear diurnal behavioral peaks and are classified into the pattern IV group. *An. saporoi*, *Oc. taeniorhynchus*, and *Oc. togoi* are unique species having different behavioral characteristics among the mosquitoes belonging to the same genus. *An. saporoi* is an anthropophilic and day-time biter whose distribution is limited to Okinawa and Iriomote Island, Japan (Toma and Miyagi, 1986). Both *Oc. taeniorhynchus* and *Oc. togoi* show high nocturnal and crepuscular activity (Omori and Fujii, 1953; Nayar and Sauerma, 1971).

3.2. Size and shape of facet lenses and rhabdoms

Facet lenses were large and almost hemispheric and the rhabdoms were conical and short in the *Anopheles* (Fig. 2A–D) and the *Culex* species (Fig. 2G–J), with the exception of *An. balabacensis* (Fig. 2E) and *An. saporoi* (Fig. 2F). Three *Ochlerotatus* species (Fig. 2M–O) appeared to belong to the group mentioned above. On the other hand, the ommatidia of *Ar. subalbatus* (Fig. 2P) and *Tr. bambusa* (Fig. 2Q) showed features that differed from that of the above group, i.e., the arc and the diameter of the facet lenses were smaller and the rhabdoms were long and cylindrical. The ommatidia of *An. balabacensis* and *An. saporoi* as well as those of *Ae. aegypti* (Fig. 2K) and *Ae. albopictus* (Fig. 2L) suggested that they could be categorized into the intermediate group between the above two groups.

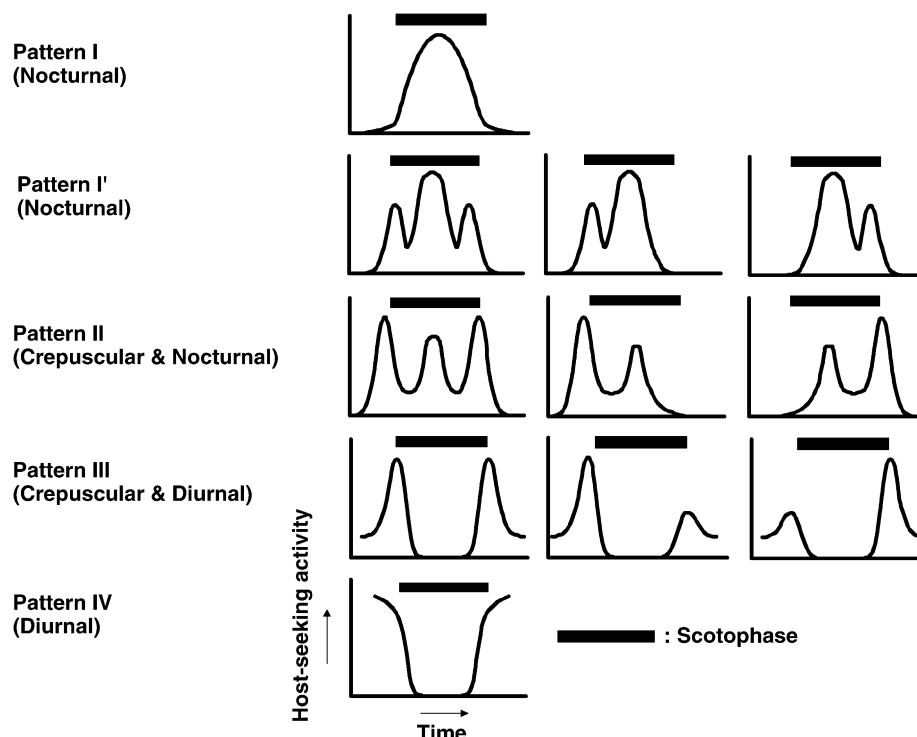


Fig. 1. Classification of host-seeking behavioral patterns of mosquitoes. Black solid bars indicate scotophase.

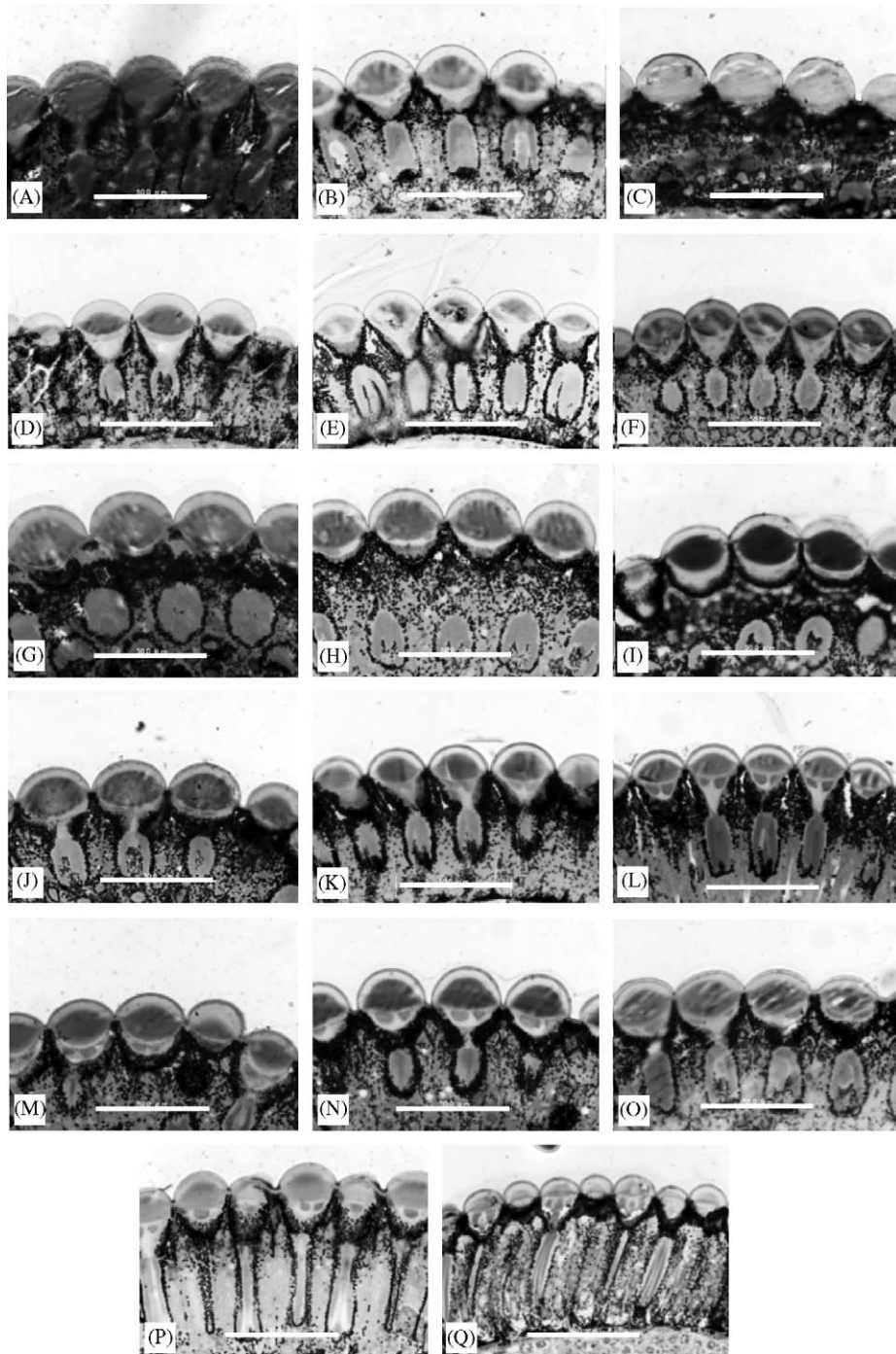


Fig. 2. Cross sections of mosquito ommatidia. White solid bars indicate 50 μm . (A) *An. albimanus*, (B) *An. stephensi*, (C) *An. dirus*, (D) *An. minimus*, (E) *An. balabacensis*, (F) *An. soperi*, (G) *Cx. quinquefasciatus*, (H) *Cx. pipiens pallens*, (I) *Cx. pipiens molestus*, (J) *Cx. tritaeniorhynchus*, (K) *Ae. aegypti*, (L) *Ae. albopictus*, (M) *Oc. togoi*, (N) *Oc. japonicus*, (O) *Oc. taeniorhynchus*, (P) *Ar. subalbatus*, and (Q) *Tr. bambusa*.

3.3. Facet diameter, interommatidial angle, and eye parameter

Facet diameters, interommatidial angles, and EPs of the mosquitoes examined in this study are listed in Table 2. The optical characteristics data of *An. gambiae* (Gliès), *Ae. aegypti*, and *Ae. albopictus* were obtained from previous studies (Clements, 1999; Kawada and Takagi, 2004).

Photoperiodic host-seeking patterns of the mosquitoes were determined from the previous reports listed in Table 2. There were minor differences in the facet diameters of *Oc. japonicus*, *Ar. subalbatus*, *Cx. pipiens pallens* in the present study (24.3, 22.6, and 23.1 μm , respectively) and those reported in the studies by Sato (1953a, 1957, 1960) (26.0, 20.0, and 23.1 μm , respectively). In conclusion, the facet diameters were large in the *Culex* and the

Ochlerotatus groups, small in two species of the *Aedes* group, and those in the *Anopheles* groups were intermediate. The *Cx. pipiens* complex is believed to be one of the major problems in mosquito taxonomy because of the lack of distinctive morphological differentiation among the complex, although each species has a markedly divergent array of physiological and behavioral traits (Fonseca et al., 2004). Considering this fact, it is interesting that there was a significant difference in the facet diameters (ANOVA, $df = 2$, $p = 0.036$) among the three assortative *Culex* species (*Cx. pipiens pallens*, *Cx. pipiens molestus*, and *Cx. quinquefasciatus*). Interommatidial angles were larger in the *Anopheles* group ($7.7\text{--}8.2^\circ$), with the exception of *An. saperoi* (4.1°), than in the *Culex* ($5.2\text{--}6.9^\circ$), *Aedes* ($5.3\text{--}6.4^\circ$) and *Ochlerotatus* ($5.1\text{--}6.5^\circ$) groups. The interommatidial angle for *Tr. bambusa* (0.96°) was exceptionally smaller than that in the other mosquito groups. There were large variations of optical characteristics depending on the mosquito species, and the EPs ranged from 0.83 to 4.2. Significant differences were observed among the EPs of the four different mosquito groups classified on the basis of photoperiodic host-seeking pattern (ANOVA, $df = 3$, $p = 0.00007$). The EP of the mosquitoes that belong to the groups of behavioral pattern I and I' were higher (EP = 2.7–4.2) than those of the mosquitoes that belong to the pattern IV group (EP = 0.8–2.3). EPs of the mosquitoes categorized in the pattern II and III groups were in the intermediate range (EP = 2.3–2.6) between the above two groups. The EPs appeared to increase as the photoperiodic host-seeking patterns shifted from IV to I (Fig. 3). The EPs for *An. saperoi* and *Oc. togoi* were exceptional and significantly different from those of other species in the same genus (ANOVA, *An. saperoi*: $df = 5$, $p < 0.001$; *Oc. togoi*: $df = 2$, $p = 0.0139$).

CVA was performed by classifying the host-seeking behavioral patterns into four categories: nocturnal (pattern I and I'), crepuscular and nocturnal (pattern II), crepus-

cular and diurnal (pattern III), and diurnal (pattern IV), which appeared to be classical categorization, as demonstrated by Edman and Spielman (1988). CVA demonstrated that the majority of total variance (69.40%) was explained by the first canonical variate in which contrast between the values of eye characteristics (i.e., facet diameter and interommatidial angle) and EP contributed most to the classification of individuals (Table 3). Plots of CVA scores showed that the centroid of the “diurnal” ellipse tended to deviate from the centroid of other groups to a great extent (Fig. 4). The morphological distance represented by the squared Euclidean distance (the square of the difference between the values of each variable) between centroid of the diurnal group and of any other group was greater than that between any other pair, with an exception of a pair involving the diurnal group (Table 4). The individuals were correctly classified into their original host-seeking behavior groups at probabilities of 72–85% using estimated discriminant functions. The proportion of the number of individuals that were classified correctly into the original groups to the total individuals

Table 3
First two canonical coefficients standardized by within variance of each eye characteristic

Characteristic	CAN 1	CAN 2
Facet diameter	2.251	1.837
Interommatidial angle	2.417	1.212
Eye parameter	−2.303	−2.505
Eigenvalue	2.445	1.069
Contribution	69.40%	30.30%

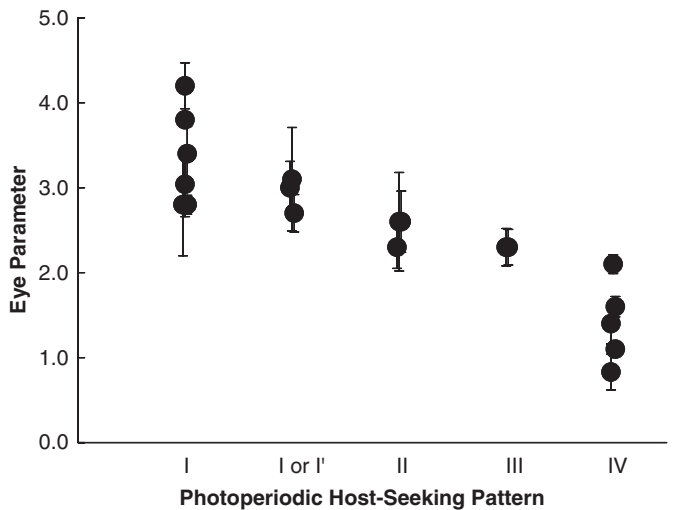


Fig. 3. Relationship between the EP and photoperiodic host-seeking pattern of mosquitoes. Each solid bar indicates 95% confidence limit.

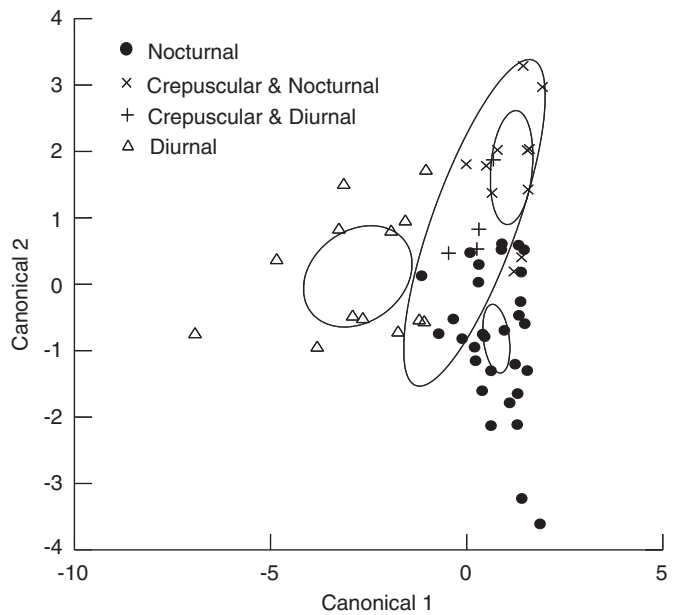


Fig. 4. Ninety-five percent confidence ellipse of the centroid of the first two canonical variate scores for each pattern of host-seeking behavior.

Table 4

Squared Euclidian distance between centroids of confidence ellipses for each different behavioral pattern

	Nocturnal	Crepuscular and nocturnal	Crepuscular and diurnal	Diurnal
Nocturnal	0.00			
Crepuscular and nocturnal	6.79	0.00		
Crepuscular and diurnal	3.36	1.59	0.00	
Diurnal	13.36	18.07	9.50	0.00

Table 5

Identification table for four characteristics of host-seeking behavioral patterns in mosquitoes

Characteristic	Nocturnal	Crepuscular and nocturnal	Crepuscular and diurnal	Diurnal
Nocturnal	22 (73.33)	2 (6.67)	6 (20.00)	
Crepuscular and nocturnal	1 (9.09)	8 (72.73)	2 (18.18)	
Crepuscular and diurnal		1 (25.00)	3 (75.00)	
Diurnal			2 (15.38)	11 (84.62)

Discriminant function for classifying individuals into each behavioral pattern was estimated by canonical variate analysis (CVA). The numbers and proportions (in parentheses) of correct identifications are shown in the diagonal positions.

Table 6

Identification table for six genera of mosquito species examined

Characteristic	<i>Aedes</i>	<i>Anopheles</i>	<i>Armigeres</i>	<i>Culex</i>	<i>Ochlerotatus</i>	<i>Tripteroides</i>
<i>Aedes</i>	5 (83.33)	1 (16.67)				
<i>Anopheles</i>	3 (13.64)	15 (68.18)	3 (13.64)	1 (4.55)		
<i>Armigeres</i>			2 (100.0)			
<i>Culex</i>		3 (18.75)	1 (6.25)	7 (43.75)	5 (31.25)	
<i>Ochlerotatus</i>			2 (20.00)	3 (30.00)	5 (50.00)	
<i>Tripteroides</i>						2 (100.00)

Discriminant function for classifying individuals into each genus was estimated by canonical variate analysis (CVA). The numbers and proportions (in parentheses) of correct identifications are shown in the diagonal positions.

was 75.86% (Table 5). However, in cases wherein the classification was based on taxonomical units, i.e., genus in this analysis, the proportion of the number of individuals that were classified correctly decreased to 62.07% (Table 6). The average proportion of the number of correctly classified individuals became much lower when the classification unit was based on “species” (36.21%, table not shown). Furthermore, MANOVA demonstrated that group differences of optical characteristics were more obvious in the case of host-seeking behavioral pattern than in the case of taxonomic status (host-seeking behavioral pattern: Wilks’ lambda = 0.006, $p = 0.0001$; genus: Wilks’ lambda = 0.15, $p = 0.0001$). These results suggest that the optical characteristics of mosquitoes would be explained better by their host-seeking behavioral patterns than by their taxonomic status.

4. Discussion

In conclusion, the relationships between the EPs for the mosquitoes and their photoperiodic host-seeking pattern reported in the present study appear to fit well with Snyder’s (1979) categorization, i.e., higher the EP, dimmer

the photo environment where the insect is most active. Land et al. (1999) reported that there were major differences in the proportions of the different ommatidial parts among the mosquitoes ranging from the nocturnal *An. gambiae* to the diurnal *Tx. brevipalpis*. They also categorized crepuscular mosquitoes, such as *Oc. detritus* (Haliday) and *Oc. punctator* (Kirby), into an intermediate group between the above two groups. In fact, the above differences were clear between *An. gambiae* and *Tx. brevipalpis* that represent the extremes of light regime. However, in their report, it was hard to distinguish the differences, with the exception of those with the maximum differences of rhabdom diameters between the crepuscular and diurnal mosquitoes. On the other hand, EPs appear to provide a satisfactorily categorization of the optical differences related to the light regime. Furthermore, the above continuum in EPs suggests that crepuscular behavior of mosquitoes is a transitional behavior in the course of evolution of nocturnal behavior to diurnal behavior.

It is noteworthy that the EPs for *An. saperoi* and *Oc. togoi* were exceptional and significantly different from those of other species in the same genus. The EP of *An. saperoi* (EP = 1.4) indicates that this species adapts to or

prefers a brighter environment than the other species in the same genus. On the other hand, the EP of *Oc. togoi* (EP = 3.0) indicates that this species prefers a darker condition than most of the other species in the same genus (*Aedes*). The above two indications of the EPs satisfactorily explain the actual differences in the photoperiodic host-seeking behavior of both species from other species in the same genus (Omori and Fujii, 1953; Toma and Miyagi, 1986). These facts suggest that the ommatidial structures of mosquitoes are not determined taxonomically but physiologically, i.e., on the basis of the type of photoenvironment in which host-seeking mosquitoes are most active.

A majority of prosimians, primates, and their ancestral insectivores are believed to be nocturnal or nocturnal in their early stages of evolution. A majority of ancestral species of the other mammals and reptiles are also believed to be nocturnal. Therefore, a majority of ancestral hematophagous insects are believed to be nocturnal, thereby functioning in parallel with the activities of their hosts. Some of the above nocturnal hematophagous insects with apposition eyes may have experienced the necessity to shift their nocturnal behavior to crepuscular or diurnal on the basis of the behavioral or environmental changes in the host animals. The uniqueness of the visual structures in *An. saperoi* and *Oc. togoi* appears to support the evidence that apposition eyes have a large adaptation range to photoenvironment. Whether the apposition eyes of mosquitoes also cause the “intra-species” differentiations among local populations of the same species that have developed specific behavioral patterns by environmental or geographical isolation or artificial selection is a matter of great interest.

Acknowledgments

We thank A. Ichinose, K. Shimabukuro, Y. Maekawa, T. Sunahara, T. Abe, and E. Kawashima, Institute of Tropical Medicine, Nagasaki University, Nagasaki, Japan; Y. Shono, Agricultural Chemicals Research Laboratory, Sumitomo Chemical Co., Ltd, Hyogo, Japan; Y. Tsuda, National Institute of Infectious Diseases, Japan; I. Miyagi and T. Toma, Faculty of Medicine, Ryukyu University, Japan; and E.J. Gerberg, Institute of Food and Agricultural Sciences, University of Florida, USA, for rearing and providing the experimental insects and for their assistance in this study.

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