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Original Article

Two-dimensional gel analysis of midgut proteins of the dengue vector *Aedes albopictus* (Diptera: Culicidae) with reference to sex and body size

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Abstract

In *Aedes* mosquito, which can vertically transmit the dengue virus to offspring, both sexes can be receptive to the virus. A number of proteins are being reported to impair the viral infection, but none of these have been isolated from the site of initial infection, the midgut. Using a two-dimensional (2-D) gel electrophoresis, we categorized proteins of the midgut from the dengue vector *Ae. albopictus* (Diptera: Culicidae) with regard to size and sex. The comparison of profiles from small and large females revealed that a set of proteins was identified as specific to large female's midguts. The comparison of profiles for males and females showed clear differences as well. A set of proteins identified in male midguts were produced in much higher quantities in females than in males. In addition, many other proteins were identified as being specifically produced only in female midguts. These results are discussed in the context of female functions.

Key words : Midgut, Proteins, Sex, Size, Dengue, *Aedes albopictus*.

Introduction

Aedes are vectors of many viral diseases impairing human development worldwide. Viruses belonging to the genus *Flavivirus*, transmitted by these mosquitoes cause dengue fever, which is being considered as one of the most re-emerging diseases. Despite the efforts made for controlling this disease, recently, dengue has increased sharply (Gubler, 1998; 2004). Facing major problems associated with lack of vaccines, insecticide resistance, environmental concerns and limited spectrum of effective insecticides, researchers combating this disease have become concerned about the need to shift to alternate control strategies.

The cycle of a salivarian transmission starts when a female ingests an infected blood meal. Dengue infection processes take place within its lumen and through the epithelium of the midgut. Thus, specific factors from the epithelium contribute much to the achievement of a productive infection. To gain entry, dengue viruses attach to target cell's surface receptors through the envelope glycoprotein of the viral membrane (Martinez-

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Barragan and del Angel, 2001). Identification of surface receptor or receptors utilized by the dengue viruses to enter into cells has been an active field. In cell lines from human, heparan sulfates (Chen *et al.*, 1997), the C-type lectins DC-SIGN and L-SIGN have been shown to mediate infection by dengue viruses (Tassaneetrithep *et al.*, 2003) and most recently, studies using a standard virus overlay protein binding assay (VOPBA) have suggested that in the liver cell line HepG2, different DENV serotypes utilize different cell surface molecules (Jindadamrongwech *et al.*, 2004a) More specifically, mass spectrometric methods have been used to identify reactive bands using VOPBA and it has been suggested that DENV-2 interacts with GRP78 (Jindadamrongwech *et al.*, 2004a) while DENV-1 interacts with the 37 kDa/67 kDa high affinity laminin receptor (Jindadamrongwech *et al.*, 2004b). With mosquito cell lines proteins interacting with dengue viruses include a 80 kDa/ 67 kDa (Munoz *et al.* 1998), a 40 kDa/45 kDa (Reyes-del Valle and del Angel, 2004) and a 37 kDa/6 kDa laminin (Tio *et al.*, 2005). Most of these studies have been based on cell lines which are physiologically unrepresentative of the midgut. This epithelium is the site of infection, development and migration (Wang *et al.*, 2001); therefore, the molecules of its epithelium may be a potential source of infection-blocking tools. In this regard, a better knowledge of midgut proteins is relevant.

In addition to the salivarian transmission, evidence also exists that the dengue virus can be vertically transmitted (Hawley, 1988; Joshi *et al.*, 2002; Mourya *et al.*, 2001), suggesting that its receptors are present in several mosquito epitheliums of both sexes. C6/36 cell lines are cells which can be easily infected by dengue virus. Such cells are derived from the asexual larval stage of *Aedes albopictus* (Skuse),

This species is increasingly gaining medical importance (Salas-Benito and del Angel, 1997). Originating in Southeast Asia, it has become well-established in Africa and the mid-east (Gratz, 2004), but being considered second to *Aedes aegypti* L. in its importance as a vector of dengue fever and dengue hemorrhagic fever in the Western Hemisphere (Reiter, 1998). Although secondary, much effort has been directed towards understanding its bio-ecology; overall, density has been reported to have a negative effect and food a positive effect on development and adult size (Mori 1979; Lord, 1998; Dieng *et al.*, 2002; Tseng, 2004), a parameter that greatly manipulates the morphological, physiological and molecular features of mosquitoes. Changes noted in blood feeding (Nasci and Mitchell, 1994), body dimensions (Timmermann and Briegel, 1999), protein content (Brogdon, 1984; Briegel, 2003), density of virus receptors (Grimstad and Haramis, 1984), infectivity (Baquar *et al.*, 1980; Grimstad and Walker, 1991; Sumanochitrapon *et al.*, 1998), enzyme synthesis (von Dungern and Briegel, 2001), and midgut thickness (Clements, 1963; Thomas *et al.*, 1993) have been attributed to size. Since total structural protein follows a highly significant and linear correlation with size (Briegel, 2003), the midgut of a large female is expected to have structurally more proteins than that of a small one. In *Ae. albopictus*, females with thick midguts exhibit higher disseminated infection rates of dengue virus-1 than those with thinner midguts (Thomas *et al.*, 1993).

The present study examines the midgut proteins of *Ae. albopictus* with regard to size and sex using a two-dimensional (2-D) gel electrophoresis. The characterization of female-specific proteins may also be useful for the identification of female-specific promoters, which could be used to drive the expression of genes encoding proteins impairing the development pathogens in transgenic mosquitoes (Grosmann *et al.*, 2001).

Materials and Methods

Mosquito and experimental specimens

The *Ae. albopictus* used here have been maintained at 26° C, 60-80% humidity under a photoperiod of 16 : 8 (L:D) for years in the Insectary of the Animal Center at Oita University, Japan. Routinely, eggs briefly kept at 13° C were hatched in deionized water. Larvae were reared at densities of 80 in 500-ml pans (length = 29 cm, width = 23.3 cm and depth = 4 cm) filled with 500 ml of old tap water and fed a mixture of yeast and fine-grained mouse pellets (1:1). Adults had access to a 4% sucrose solution and females were routinely fed for an hour on anaesthetized mice approximately 5-days post-emergence.

To obtain large and small female adult mosquitoes, we referred to McCombs (1980) and Grimstad and Haramis (1984). Overall, high and low larval densities were fed with respectively low and high food amounts as shown in **Table 1.** Adults were sugar-fed with an apparatus consisting of a 250 ml plastic container covered with a piece of aluminum foil at the centre of which was opened a hole filled with wick connecting the solution to a covering cotton pad. After 4 days of

Table 1 Feeding regimens for the production of males different sized females of Aedes albopictus

	Low larval density[1]			High larval density[1]		
Days	Yeast/MP[3]	TetraMin®	Medium	Yeast/MP[3]	TetraMin®	Medium
0[4]	12	3		3	0	
2	16	4	renewed	4	0	
4	20	4		5	0	
6	28	4		7	0	
8	28	4		7	0	

1) Larval densities (40/500 mℓ)
2) Larval densities (80/500 mℓ)
3) MP: Mouse pellets (mg)
4) Day 0 corresponds to the day eggs hatching and set up.

sugar feeding, adult females were randomly sampled from the two larval treatments and were measured their body size.

Dissection

Midguts of both sexes of adults deriving from each larval regime were dissected under a binocular microscope (Nikon Type 102 S10X, Japan) in droplets of cool phosphate-buffered saline (PBS). Forceps and pins were changed for every each dissection. The damaged midguts were discarded, whereas the intact ones were stored at -80 ° C for 2-D gel electrophoresis analysis.

Protein analysis

We performed a 2-D gel electrophoresis analysis as follows: 200 μl of buffer (1.6 mg of DTT, 3 μl ampholyte (pH 3-7), 10 μl 0.1% bromophenol blue, 8 M urea, 4% chaps; final volume: 10 ml) was mixed with a midgut. The resulting solution was moved to a tray and there was covered with a stripe gel. After an hour, we added 800 μl of mineral oil to the midgut solution prior to the first dimension at 19/24℃ (rehydration condition) using the Protean IEF Cell (Bio-Rad Laboratories, California, USA). After 12 hours, the stripe gels were rinsed with 250 ml of two types of buffer (buffer I: 20 ml 6 M urea, 2% SDS, 0.375 M Tris-HCl, 20% glycerol and 2% w/v DTT; and buffer II: 20 ml 6 M urea, 2% SDS, 0.375 M Tris-HCl, 20% glycerol and 0.5 g iodoacetamide). The second dimension was then performed using 12.5% polyacrylamide Ready Gels J (Bio-Rad Laboratories, California, USA), which was cemented with the stripe using a 1% dyed agarose gel solution. 2 μl of Precision Plus Proteins™ (Bio-Rad Laboratories, California, USA) was used as a marker. Electrophoresis conditions were 40 mA, 80 volts for an hour and 30 minutes. Silver staining was performed according to the manufacturer's (2D-Silver stain II "Daiichi", Daiichi Pure Chemicals CO., Ltd, Tokyo, Japan) specifications.

Determination of protein concentration

Midgut protein concentrations expressed as optical densities were determined in triplicate for large males, large females and small females based on Lowry-SDS method from the Ultrospec 300 UV/Visible spectrophotometer (Pharmacia Biotech ltd, Cambridge, UK) fixed at 750 nm wavelength. The RC DC protein assay (Bio-Rad Laboratories, California, USA) was performed with a standard curve based on 6 dilutions of bovine serum albumin (BSA) standard from 0.25 mg/ml to 1.5 mg/ml.

Data collection and analysis

Wing length considered as alternative index of body size was measured from the axillary incision to the distal end excluding the fringe according to von Dungern and Briegel (2001). To find out whether there was variability in size and protein content among the groups, we calculated the coefficient of variation by dividing the standard errors by the mean sizes and mean protein concentrations. Analysis of variance was performed using SYSTAT®11 statistical software package (SYSTAT®11 DATA, 2004) to compare size and protein concentrations.

The stained gels were photographed (Dimage Xg Konica Minolta Zoom Lens 5.7-17.1 mm, Tokyo, Japan), the photos computerized, and the image controller fixed at a contrast of 52% and at lightness of 50% and analyzed by eye. We analysed qualitatively the effects

of size by comparing profiles of midguts from small and large females and the effects of sex using those from large-sized male and female adults. Profiles were compared based on the presence/absence and the darkness/lightness of spots. The presence of a spot means an expression of a protein, whereas its absence, a repression. The dark aspect of a given spot means a high expression level, whereas its light aspect means a low level of expression. Due to the high number of spots, we proceeded as follows: (1) for males and females, which were very different in spot number, the comparison was based on the detection of at least 15 shared spots and 25 spots that were specific to the female; (2) for small and large females, which had fewer differences, the comparison was based on the detection of at least 15 shared spots and at least 5 spots that were specific to the large females. Differences of patterns were considered if observed in at least two replicates. In all analyses only differences related to the absence/presence of spots and/or the differences in darkness between identical spots were taken into account.

Results

Breeding outcome

Body size varied significantly with adult physiological status ($F = 118.3$, $df = 2$, $P < 0.005$). Males (2.4 ± 0.067 mm: Mean ± SD) derived from larvae raised under a low density were smaller than their female counterparts (3.4 ± 0.027 mm), which in turn were larger than females (2.9 ± 0.051 mm) derived from larvae reared under a high density (**Fig. 1**). The (2.4 ± 0.067 mm), (3.4 ± 0.027 mm) and (2.9 ± 0.051 mm) mosquitoes were referred to respectively as "large males", "large females" and "small females". The coefficient of variation of size was 0.03 for the males, 0.008 for large females and 0.017 for the small females.

Protein concentration

Protein concentrations were quantified in triplicate for each midgut physiological status. The concentrations were undeterminable by the Albumin standard because they were out of its range. Therefore, we used the optical densities to calculate the concentrations. There was a significant difference in protein content between the different midgut statuses ($F = 12.1$, $df = 2$, $P = 0.008$). Midguts from the large females had greater concentrations of proteins than those of the large males, which in turn had greater concentrations than the small females (**Table 2**).

Table 2 Concentration of proteins in Aedes albopictus midguts with different physiological status

Physiological status	Mean concentration (mg/ml)
Large male	0.167 ± 0.007^{c}
Large female	0.217 ± 0.009^{a}
Small female	0.190 ± 0.006^{b}

By ANOVA, values with the same letter do not show a significant difference ($P<0.05$).

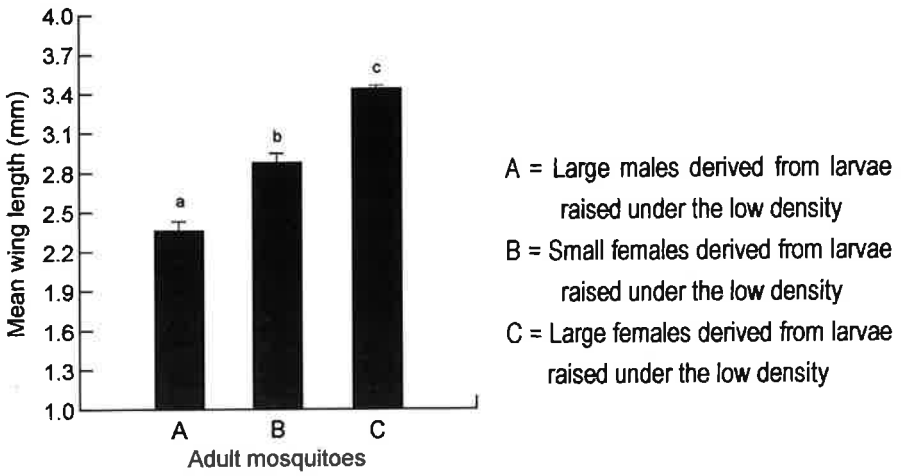

Fig. 1 Body size of the experimental mosquitoes. By ANOVA, values with the same letter do not show a significant difference ($P<0.05$).

Body size and midgut proteins

Figure 2 shows 2-D silver-stained gels of *Ae. albopictus* midgut proteins of a large (A) and a small (B) females. The large female derived from a well-fed larva and its small counterpart from a poorly-fed larva. The sign (+) indicates a high level of expression and (−) a low level of expression. SL means specific to large female. Protein profiles varied clearly with female size. The profiles of midguts from large females showed a slightly higher number and darker spots. Large females expressed 5 specific proteins (SL1, SL2, SL3, SL4 and SL5) and over-expressed 20 others shared with the small females. Clearly, the midguts of large females contained a greater number of proteins than that of the small ones (**Fig. 2**).

Sex and midgut proteins

Figure 3 shows 2-D silver-stained gels of *Aedes albopictus* midgut proteins of male (A) and female (B). Both were well-fed as larvae and sugar-fed as adults. The sign (+) indicates a high level of expression and (−) a low level of expression. SF means specific to female. The comparison of protein profiles from males and females showed clear differences in patterns of expression. Females had far a greater number and stronger protein spots. At least 26 protein spots were

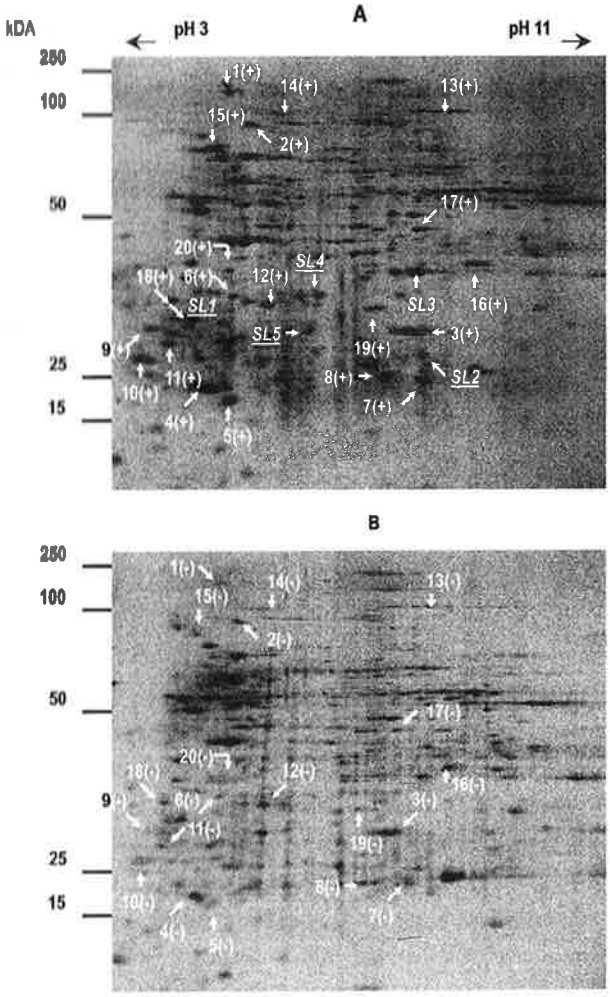

Fig. 2 Two-dimensional (2-D) silver-stained gels of *Aedes aalbopictus* midgut proteins of a large (A) and a small (B) females. The large female derived from a well-fed larva and its small counterpart from a poorly-fed larva. At emergence, both had access to a sugar meal. The sign (+) indicates a high level of expression and (−) a low level of expression. SL means speciffic to large female.

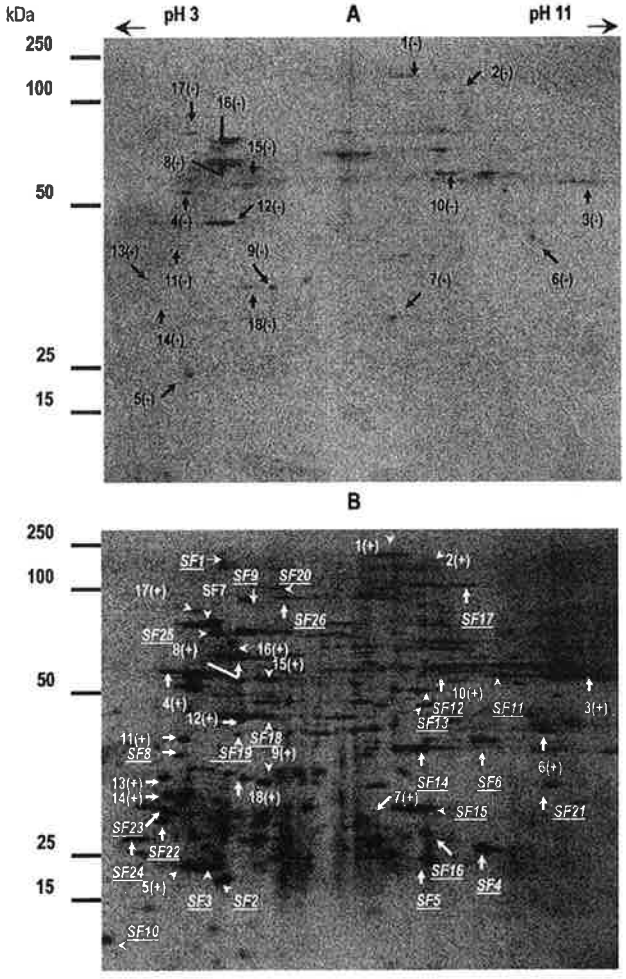

Fig. 3 Two-dimensional (2-D) silver-stained gels of *Aedes aalbopictus* midgut proteins of male (A) and female (B). Both were well-fed as larvae and sugar-fed as adults. The sign (+) indicates a high lecel of expression and (−) a low level of expression. SF means speciffic to female.

absent in the profiles of males (**Fig. 3**A) and present in those of females (**Fig. 3**B: spots SF1 to SF26). In addition, at least 17 spots shared between the two profiles were darker in that of females (**Fig. 3**A). These differences in spot patterns indicate that the midguts of females contained more proteins than those of males. Apart from specific proteins (SF1 to SF26), female midguts produced higher amount of proteins they shared with males. These differences in protein profiles clearly suggest either morphological or physiological discrepancies between the two sexes.

Discussion

The rearing procedure adopted produced different specimens. In particular, there was a marked difference in size between the female groups. Larvae reared under uncrowded populations (40 larvae in 500 ml of water) produced larger females as often as those raised under crowded conditions (80 larvae in 500 ml of water). Variability in size expressed as a coefficient of variation was low within each group when referring to a report from Siegel *et al.* (1994). Populations with a coefficient of variation of 2% have little variation. However, populations with a coefficient of variation of 10% have a high variation. This is likely to suggest that fitness was similar between individuals within each group used in the present study.

Midgut protein profiles significantly varied between the large and small females. In accordance with results on protein concentration, large female midguts contained more proteins than small female midguts. In addition, they over-produced most proteins shared with small females. These observations are in line with reports correlating size to biomass. In the present study, the large adults were derived from well-fed larvae and their small counterparts from poorly-fed ones. Probably the larger females have thicker midguts and consequently greater numbers of proteins interacting with the virus as already proposed by Grimstad and Haramis (1984). Thus, it appears clear that size needs to be considered in molecular investigations in order to avoid misleading conclusions.

Major differences in protein content and expression were also observed between male and female of *Ae. albopictus*. At least 17 proteins were only found in the midguts of females, which also highly produced most of those found in male midguts. Such sex-related differences in midgut proteome have been well documented. Cazares-Raga *et al.* (1998) and Prevot *et al.* (2003) investigating the protein expression pattern at the midgut level, reported greater expressions in females of *An. gambiae*. Males and females do not have the same morphological features. Male midguts resemble those of the females, consisting of columnar and regenerative cells; however, males have no spot desmosomes and the grid-like substructure of the basement membrane (Clements, 1992). Apart from these cellular/structural differences, sex-related variability in midgut proteome has been related to the physiological roles of males and females after emergence (Prevot *et al.*, 2003). Moreover, in nature both sexes feed on nectar (Clements, 1992), thus indicating that they share a similar physiological function, which is to process sugared meals. But in addition, a female must digest blood and ensure defensive reactions against possible pathogens. This requires structurally adapted morphological features different from those of a male. In *Ae. aegypti*, the female has more rough endoplasmic reticulum than the male, due probably to the fact that females can synthesize enzymes for blood digestion, which are absent in males. The female's functions are also reflected by the high presence of organelles and membrane systems such as mitochondria and basal labyrinth (Rudin and Hecker, 1976). Here, we have used sucrose solution as the feeding resource for both sexes. This strongly suggests that sugar processing was the sole function activated in our experimental specimens. It is well-known that a male does not feed on blood. With reference to these statements, it is likely that the 17 proteins specific to females and those of males that they over-produced are either structural or involved in functions other than sugar processing.

The present study demonstrates that the protein content of the midgut of a large bodied female is different from that of the midgut of a small female. Large size has been often shown to have a direct effect on infection (Grimstad and Walker, 1991; Sumanochitrapon *et al.*, 1998) except in the *Ae. triseriatus*-LaCrosse encephalitis system (Grimstad and Haramis, 1984). The results on midgut and size suggest that a difference may arise in receptivity to infection. The results from Thomas *et al.* (1993) showing clear evidence of a greater dissemination rate of the dengue virus-1 in the females with thick midgut than those with thinner midgut likely support our contention. Our observations suggest a better consideration of size in molecular approaches of female

physiology. In addition, we demonstrated that the male midgut proteins is fundamentally different from that of females, despite the shared trait of processing sugar meals, and that female midguts had more proteins, which are probably involved in its secondary functions. Further studies involving blood feeding and infection are needed to screen these functions.

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References

Baqar, S., C.G. Hayes, and J. Ahmed (1980) The effect of larval rearing conditions and adult age on the susceptibility of *Culex tritaeniorhynchus* to infection with West Nile virus. *Mosquito News* 40 : 165－171.

Briegel, H. (2003) Physiology bases of mosquito ecology. *J. Vector Ecol.* 28 : 1－11.

Brogdon, W. G. (1984) Protein levels in *Anopheles albimanus* are positively correlated with body weight. *Comp. Biochem. Physiol.* 79 : 457－459.

Cazares-Raga, F. E., M. E. Sanchez-Contreras, M. H. Rodriguez, and F. C. Hernandez-Hernandez (1998) Sex-specific proteins and proteases present in the midguts of *Anopheles albimanus* (Diptera: Culicidae). *J. Med. Entomol.* 35 : 184－186.

Chen, Y., T. Maguire, R. E. Hileman, J. R. Fromm, J. D. Esko, R. J. Linhardt, and R. M. Marks (1997) Dengue virus infectivity depends on envelope protein binding to target cell heparan sulfate. *Nat Med.* 3 : 866－871.

Clements, A. N. (1963) *The physiology of mosquitoes. Vol. 1.* pp. 393. Pergamon Press, London.

Clements, A. N. (1992) *The biology of mosquitoes. Vol. 1.* pp. 509. Chapman & Hall, London.

Dieng, H., C. Mwandawiro, M. Boots, R. M. Morales, T. Satho, N. Tuno, Y. Tsuda, and M. Takagi (2002) Leaf litter decay process and the growth performance of *Aedes albopictus* larvae (Diptera: Culicidae). *J. Vector Ecol.* 27 : 31－38.

Gratz, N. G. (2004) Critical review of the vector status of *Aedes albopictus*. *Med. Vet. Entomol.* 18 : 215－227.

Grimstad, P. R. and L. D. Haramis (1984) *Aedes triseriatus* (Diptera: Culicidae) and La Crosse virus III. Enhanced oral transmission by nutrition-deprived mosquitoes. *J. Med. Entomol.* 21 : 249－256.

Grimstad, P. R. and E. D. Walker (1991) Aedes triseriatus (Diptera: Culicidae) and La Crosse virus IV. Nutritional deprivation of larvae affects the adult barriers to infection and transmission. *J. Med. Entomol.* 28 : 378－386.

Grossman, G. L, C. S. Rafferty, J. R. Clayton, T. K. Stevens, O. Mukabayire, and M. Q. Benedict (2001) Germline transformation of the malaria vector, *Anopheles gambiae*, with the piggyBac transposable element. *Insect Mol. Biol.* 10 : 597－604.

Gubler, D. J. (1998) Dengue and dengue hemorrhagic fever. *Clin. Microbiol. Rev.* 11 : 480－496.

Gubler, D. J. (2004) The changing epidemiology of yellow fever and dengue, 1900 to 2003: full conclusion. *Comp. Immunol. Microbiol. Infect. Dis.* 27 : 319－330.

Hawley, W. A. (1988). The biology of *Aedes albopictus*. *J. Am. Mosq. Control Assoc.* Suppl. no. 1 : 1－40.

Jindadamrongwech, S. and D. R. Smith (2004a) Virus Overlay Protein Binding Assay (VOPBA) reveals serotype specific heterogeneity of dengue virus binding proteins on HepG2 human liver cells. *Intervirol.* 47 : 370－373.

Jindadamrongwech, S., C. Thepparit, and D. R. Smith (2004b) Identification of GRP 78 (BiP) as a liver cell expressed receptor element for dengue virus serotype 2. *Arch. Virol.* 149 : 915－927.

Joshi, V., D. T. Mourya, and R. C. Sharma (2002) Persistence of dengue-3 virus through transovarial transmission passage in successive generations of *Aedes aegypti* mosquitoes. *Am. J. Trop. Med. Hyg.* 67 : 158－161.

Lord, C. C. (1998) Density dependence in larval *Aedes albopictus* (Diptera: Culicidae). *J. Med. Entomol.* 35 :

825－829.

Martinez-Barragan, J. J. and R. M. del Angel (2001) Identification of a putative co-receptor on vero cells that participate in dengue-4 virus infection. *J. Virol.* 75 : 7818－7827.

McCombs, S. D. (1980) Effect of differential nutrition of larvae on adult fitness of *Aedes triseriatus*. pp. 123. M.S. Thesis, Univ. Notre Dame, Indiana.

Mori, A. (1979) Effects of larval density and nutrition on some immature and adults *Aedes albopictus*. *Trop. Med.* 21 : 85－103.

Mourya, D. T., Gokhale, A. Basu, P. V. Barde, G. N. Sapkal, V. S. Padbidri, and M. M. Gore (2001) Horizontal and vertical transmission of dengue virus type 2 in highly and lowly susceptible strains of *Aedes aegypti* mosquitoes. *Acta Virol.* 45 : 67－71.

Munoz, M. L., A. Cisneros, J. Cruz, P. Das, R. Tovar, and A. Ortega (1998) Putative dengue virus receptors from mosquito cells. *FEMS Microbiol. Let.* 168 : 251－258.

Nasci, R. S. and C. J. Mitchell (1994) Larval diet, adult size, and susceptibility of *Aedes aegypti* (Diptera: Culicidae) to infection with Ross River virus. *J. Med. Entomol.* 31 : 123-126.

Prevot, G. I., C. Laurent-Winter, F. Rodhain, and C. Bourgouin (2003) Sex-specific and blood meal-induced proteins of *Anopheles gambiae* midguts: analysis by two-dimensional gel electrophoresis. *Malaria* J. 2 : 1.

Reiter, P. (1998) *Aedes albopictus* and the world trade in used tires, 1985-1995: the shape of things to come? *J. Am. Mosq. Control Assoc.* 14 : 83－94.

Reyes-del Valle, J. and R. M. del Angel (2004) Isolation of putative dengue virus receptor molecules by affinity chromatography using a recombinant E protein ligand. *J. Virol. Met.* 116 : 95－102.

Rudin, W. and H. Hecker (1976) Morphometric comparison of the midgut epithelial cells in male and female *Aedes aegypti* L. (Insecta, Diptera). *Tissue Cell* 8 : 459－470.

Salas-Benito, J. S. and R. M. del Angel (1997) Identification of two surface proteins from C6/36 cells that bind dengue type 4 virus. *J. Virol.* 71 : 7246－7252.

Siegel, J. P., R. J. Novak, and W. G. Ruesink (1994) Relationship between wing length and dry weight of mosquitoes. *J. Am. Mosq. Control Assoc.* 10 : 186－196.

Sumanochitrapon, W., D. Strickman, R. Sithiprasasna, P. Kittayapong, and B. L. Innis (1998) Effect of size and geographic origin of *Aedes aegypti* on oral infection with dengue-2 virus. *Am. J. Trop. Med. Hyg.* 58 : 283－286.

Tassaneetrithep, B., T. H. Burgess, A. Granelli-Piperno, C. Trumpfheller, J. Finke, W. Sun, M. A. Eller, K. Pattanapanyasat, S. Sarasombath, D. L. Birx, R. M. Steinman, S. Schlesinger, and M. A. Marovich (2003) DC-SIGN (CD209) mediates dengue virus infection of human dendritic *cells. J. Exp. Med.* 197 : 823－829.

Thomas, R. E., W. K. Wu, D. Verleye, and K. S. Rai (1993) Midgut basal lamina thickness and dengue-1 virus dissemination rates in laboratory strains of *Aedes albopictus* (Diptera: Culicidae). *J. Med. Entomol.* 30 : 326－331.

Timmermann, S. E. and H. Briegel (1999) Larval growth and biosynthesis of reserves in mosquitoes. *J. Insect Physiol.* 45 : 461－470.

Tio, P. H., W. W. Jong, and M. J. Cardosa (2005) Two dimensional VOPBA reveals laminin receptor (LAMR1) interaction with dengue virus serotypes 1, 2 and 3. *Virol. J.* 2 : 25.

Thomas, R. E., W. K. Wu, D. Verleye, and K. S. Rai (1993) Midgut basal lamina thickness and dengue-1 virus dissemination rates in laboratory strains of *Aedes albopictus* (Diptera: Culicidae). *J. Med. Entomol.* 30 : 326－331.

Tseng, M. (2004) Sex-specific response of a mosquito to parasites and crowding. *Proc. Biol. Sci.* 271 (Biol. Let. Suppl. 4): S186－S188.

von Dungern, P. and H. Briegel (2001) Protein catabolism in mosquitoes: ureotely and uricotely in larval and imaginal *Aedes aegypti. J. Insect Physiol.* 47 : 131－141.

Wang, P., J. T. Conrad, and M. Shahabuddin (2001) Localization of midgut-specific protein antigens from *Aedes aegypti* (Diptera: Culicidae) using monoclonal antibodies. *J. Med. Entomol.* 38 : 223－230.

デング熱媒介ヒトスジシマカ（双翅目：カ科）の性別と体の大きさに関連した中腸タンパク質の二次元ゲル解析

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サルモン コマラミスラ[4]．牛島廣治[5]．高崎智彦[6]．倉根一郎[6]．江下優樹[1]

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デングウイルスを子孫に垂直伝播可能なヤブカ類では，雌雄いずれも本ウイルスに感受性をもつ．デングウイルスの感染により，多くのタンパク質の機能が損なわれるという報告がある．しかし，これらタンパク質は，最初の感染部位である中腸組織から，いずれも分離されていない．我々は，２次元ゲル電気泳動を用いて，デング熱媒介ヒトスジシマカ成虫の体の大きさと性別に関連した中腸タンパク質の特徴を調べた．雌蚊体の大小比較では，一群のタンパク質が両者に共通に見られ，その量は大きな雌蚊に多く観察された．また，これらとは異なる一群のタンパク質が，体の大きな雌蚊の中腸に特異的に認められた．蚊の雌雄間においても中腸タンパク質に明確な違いが認められた．つまり，雄の中腸で認められた一群のタンパク質は，雌の中腸においても同様に認められたが，その生産量は雄よりも多かった．さらに，これらのタンパク質に加えて，雌の中腸内でのみ特異的に産生される一群のタンパク質が観察された．本報告は，以上の結果から，雌蚊が持つ生理的機能との関連について考察した．

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Original Article

Species composition and the vertical niche breadth of ground beetles (Carabidae, Brachinidae) in the Southern Japan Alps

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Abstract

Field studies were conducted in the Southern Japan Alps from June to September of 2001 in order to clarify the species composition and vertical distribution of ground beetles. Eleven survey sites ranging in altitude from 1000 m to 2600 m were selected at Mt. Senjo. In June of 2001, pitfall traps were set 3 times at survey sites from 1000 m to 1400 m in altitude, and from August to September of 2001, trap collection was carried out twice at altitudes from 1000 m to 2600 m. All traps were baited with a lactic acid beverage mixed with 70% ethyl alcohol. A total of 2337 individuals comprising 37 species of ground beetles of Carabidae and Brachinidae were collected in this study. The dominant species found in the June collections were *Pterostichus subovatus* (44.9% of the total) and *Leptocarabus procerulus* (38.6% of the total). The 4 most numerous species of the individuals collected in August and September were *Leptocarabus arboreus horioi, Trigonognatha aurescens, Pterostichus brunneipennis akaishicus* and *Synuchus cycloderus*, which represented 76.3% (1649 individuals) of the total collected. The vertical niche breadths of ground beetles were calculated using the data of the August and September collections. The 5 species which showed the highest values for niche breadth were *S. cycloderus, Pt. brunneipennis akaishicus, L. arboreus horioi, P. aeneola* and *Synuchus melantho*. *S. cycloderus* showed a wide vertical distribution from 1000 m to 2400 m and also showed the highest value of niche breadth. Species of high relative abundance had wide niche breadth though we found no relationship between niche breadth and relative abundance among species of low abundance. The correlation coefficient between niche breadth and mean altitude of the vertical distribution indicated a slightly positive relationship. We include a discussion of the selection of appropriate species to represent the mountainous environment.

Key words : Southern Japan Alps, Ground beetles, Vertical distribution, Pitfall trapping, Niche breadth

Introduction

Many researchers have studied the species composition, seasonal abundance and distribution of ground beetles in various habitats (Thiele, 1977; Luff, 1987; Suttiprapan *et al.*, 2003; French *et al.*, 2004; Siddiquee *et al.*, 2005), and ground beetles have been selected as indicator insects of various environments (Oshitani, 1996; Allegro and Sciaky, 2003) because of the requirements of wide distribution, sensitivity to environmental variation and a standard sampling method (pitfall trapping) (Dufrêne *et al.*, 1990; Sunose, 1992). Additionally, several endangered ground beetles are important species with respect to environmental impact assessment.

The environmental conditions of mountainous areas differ from those of non-mountainous areas not only in their altitude but also in their faunas, and there is great diversity in the insect species that live and procreate in various mountain habitats. Recently, the environments of high mountainous areas have been disturbed by human activity, such as the construction of a dam or road, and the overuse of famous beauty spots. Ground beetles show a strong relationship with environmental conditions and act as potential bioindicators of stability or the degree of ecosystem stress, provided that their relative abundance can be assessed (Allegro and Sciaky, 2003). However, there

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impact assessment.

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Mt. Senjo is located in the Southern Japan Alps, which extend across Nagano, Yamanashi and Shizuoka Prefectures in the middle part of Japan and are host to various kinds of alpine plants, wild animals and insects. In 1952, construction was begun on the South Alps forest road, which opened to the Kitazawa Pass through the national park of the Southern Japan Alps in 1980. Martin (1989, 1992) recorded approximately 200 species of beetles collected by pitfall traps at Mt. Senjo and Mt. Kaikomagadake; after Martin's studies, there have been some reports on ground beetles (Tahira, 1995; Morita, 1998) but no reports on species composition or abundance in this area. In 2004, several carabid species inhabiting only the area around Mt. Senjo were designated as endangered species (Nagano Nature Conservation Research Institute, 2004).

In the present study, in order to clarify the species composition and vertical distribution of ground beetles of Carabidae and Brachinidae in the Southern Japan Alps, field studies were conducted from June to September of 2001 for the purpose of selecting appropriate species to represent the mountainous environment.

Materials and Methods

Study sites

Eleven sites were selected at different altitudes on Mt. Senjo in the Southern Japan Alps. A map of the survey sites is shown in **Fig. 1**. Below 1500 m, deciduous broad-leaved trees were dominant, and coniferous trees including Marie's fir (*Abies mariesii*), silver fir (*Abies veitchii*) and Japanese hemlock (*Tsuga diversifolia*) were dominant from 1500 m to 2700 m; above 2700 m, the Japanese mountain stone pine (*Pinus pumila*) was dominant.

Pitfall trapping was conducted a total of 5 times from June to September of 2001 (**Table 1**). The high areas of Mt. Senjo were still covered with snow in June, and pitfall traps were therefore set only at survey sites from 1000 m to 1400 m. In the months of July to September, trap collections were carried out twice at altitudes ranging from 1000 m to 2600 m.

Study methods

Transparent plastic cups 13 cm deep with upper and lower diameters of 9 and 6 cm, respectively, were used as traps. All traps were baited with a lactic acid beverage (Calpis™, Calpis Co., Ltd., Tokyo, Japan) mixed with 70% ethyl alcohol as a preservative material necessary due to the long interval between setting the traps and collecting the insects. Each trap was placed into the ground and covered by a stone or a piece of wood. Covering the traps provided favorable shade for ground beetles, reduced evaporation of the ethyl alcohol and prevented excess rainwater and small mammals from entering the traps.

Seven pitfall traps were placed at each study site from 1000 m to 1400 m and 5 pitfall traps at each site above 1600 m. The survey dates and numbers of traps at each survey site are given in **Table 1**. The traps were sometimes destroyed by animals, so the number of traps upon collection was lower than the number initially set. The names and numbers of the species of ground beetles captured in the traps were recorded. In the present study, the name *Pristosia colpodoides* was regarded as a synonym of *Pristosia aeneola*.

Niche breadth

The niche breadth (B_I) of a species i with respect to a given environmental factor can be defined as follows (Kobayashi, 1995):

$$B_I = -\sum p_{ij} \cdot \ln p_{ij}$$

$$p_{ij} = n_{ij} / \sum n_{ij}$$

where p_{ij} is the proportion of individuals collected at the jth study site in the total sample of species i and n_{ij} is the number of trapped species i at the jth study site. In the present study, we calculated the niche breadth of ground beetles with respect to altitude using the survey data collected in August and September of 2001.