Comparative Mating Flight Times of Asian Honey Bees

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SUMMARY

We compare data from six studies on the timing of drone flights of different assemblages of *Apis* species from different regions of Asia. Two major results are evident. First, there is extensive intraspecific variation in the temporal occurrence of drone flights between sites, suggesting that the timing of mating flights can be altered by selective forces. Second, there is almost no overlap in the flight distributions of sympatric species within a locality. This pattern is suggestive of reproductive character displacement through reinforcement, which would require selection against interspecific hybrids. Alternatively, the basis for the pattern of non-overlapping drone flight distributions may be the attraction that drones of all species have to the sex pheromone compound 9-ODA, which may result in interspecific interference and a reduction in the efficiency of mating.

INTRODUCTION

There are at least eight species of honey bees in Asia (Otis, 1991, 1996; Tingek *et al.*, 1996), including two pairs of closely related species (e.g., *Apis cerana* and *A. nigrocincta* in Sulawesi; *A. cerana* and *A. nuluensis* in Borneo). Up to four species live sympatrically (e.g., in the same geographic area). This creates situations in which interspecific interactions could occur, such as competition for food, competition for nest sites (for cavity-nesting species), and interactions at the time of mating. Interspecific contact between queens and drones of different species could delay mating (cf. Ruttner and Maul, 1983) or result in interspecific crosses and the consequent production of inviable zygotes or offspring with reduced fitness.

All *Apis* species have fundamentally similar mating systems. Drones and queens fly from colonies and are believed to encounter each other in drone congregation areas; mating flights are restricted to a portion of

the available flight period; drones are attracted to queens chemically (i.e., 9-keto-2(*E*)-decenoic acid, or 9-ODA, seems to serve as a mating attractant in all species; Free 1987); and queens mate with several drones on a single mating flight. By virtue of being different species, there are barriers to interspecific mating (reproductive isolating mechanisms, or RIMs). In the case of honey bees, the possible premating RIMs are seasonality and timing of mating, location of mating, species-specific mating attractants (pheromones) of queens, sexual selection by queens of specific drones, and genital morphology that prevents physical coupling (Otis & Hadisoesilo, 1999; Koeniger & Koeniger, in press). Potential postmating RIMs include reduced hybrid fitness and sterility/infertility of hybrid offspring.

Koeniger and Wijagunasekera (1976) first drew attention to the temporal segration of mating flights of sympatric honey bee species. Similar studies have followed in other regions, and it is now possible to compare the timing of mating flights of different honey bee species in several geographic locations. We compare these data, make several generalisations, and discuss processes that may have caused the observed patterns.

METHODS

Data on timing of mating flights were obtained from published reports for Thailand, Borneo, Sri Lanka, Peninsular Malaysia, Sulawesi, and Japan. The legend of figure 1 provides more details on these localities and the sources of the data.

Most authors have reported times as observed in the local time zone of the study site. In such cases, we converted the local times to solar zenith times by adding (or subtracting) 4 minutes for each 1° of longitude east (west) of the meridian in the center of the time zone (e.g., 75°E, 90°E, 105°E). In the case of the Thai data, the appropriate conversion required subtracting 12 minutes from each observation, not adding 12 minutes as Rinderer *et al.* (1993) did. Consequently, we corrected the Thai data by subtracting 24 minutes. Differences between studies in the recording of data (time of departure; time of entry; combined departure and entry times) introduced minor errors that could not be corrected. Because daylength varies according to date and latitude, the approximate time of sunset was determined from Table 171 of List (1968).

RESULTS

The temporal distributions of drone mating flights from the six geographic localities are shown in Figure 1. Two striking results emerge. First, mating flight times are generally not species specific. For example, in Thailand where both *A. andreniformis* and *A. florea* occur, the peak time of the 2.5 hr flight period of *A. florea* drones is at 14.30 h, whereas in Sri Lanka, where *A. andreniformis* does not occur, the *A. florea* drones fly about an hour earlier (peak flight: 13.30 h) and longer (3.0 hr). Even more striking are the data for *A. cerana*. In Japan where it is the only endemic species of *Apis*, the drone flight period lasts 3.5 hr with a peak at 15.20 h. Elsewhere, with from 2-4 sympatric *Apis* species present, the flight periods are generally shorter (2.0-2.5 h), and the peak of drone flight varies by more than three hours (Sulawesi: 13.40 h; Borneo: 14.20 h; Malaya: 14.55 h; Thailand: 16.05 h; Sri Lanka: 16.55 h). Only drones of *A. dorsata* had a consistent flight period that always occurred for a short interval of time after sunset.

Second, there is very low overlap between drone flight distributions of species within a site. In Sri Lanka, the Malay Peninsula, and Sulawesi, there is essentially no overlap between the flight periods of the three species present in each locality (data for *A. koschevnikovi* from Malaya are lacking). In Borneo, the apparent overlap in flight periods of *A. nuluensis* and *A. andreniformis* is misleading because the two species live at different elevations, with *A. andreniformis* below 1600m (Otis, 1996), and *A. nuluensis* generally above 1800 m (Tingek *et al.*, 1996). The apparent overlap indicated by Koeniger *et al.* (1988, 1996) for drones of *A. koschevnikovi* and *A. dorsata* in Borneo is an artifact of reporting data in 15 minute intervals. The only species whose drone flights overlap extensively are *A. florea* and *A. cerana* in Thailand (Rinderer *et al.*, 1993).

DISCUSSION

The high intraspecific variability in the timing of drone flights between locations (most notable in *A. cerana* but evident in other species as well) suggests that this aspect of the mate recognition system of honey

bees can be easily adjusted evolutionarily. Genetic factors are known to affect the timing of mating flights (Rowell *et al.*, 1986; Koeniger *et al.*, 1994). Only minor genetic changes should be necessary to alter the pacemakers that regulate circadian rhythms (Hall, 1998) and probably regulate the timing of honey bee mating flights, resulting in a shift of drone and queen flight distributions so that they no longer overlap with those of sympatric species. Estimates of the length of time that species have resided in a particular location derived from the degree of overlap of drone flight distributions (e.g., Koeniger & Wijayagunasekera, 1976; Rinderer *et al.*, 1993) should be interpreted with caution because the timing of mating flights seems to be easily altered. Furthermore, there are other components of the mate recognition system of honey bees that differ between species (reviewed by Otis & Hadisoesilo, 1999). Ecological factors (e.g., predators) also probably influence the timing of mating flights

It has generally been observed, both in models (e.g., Gavrilets & Boake, 1998) and in empirical studies (Giddings *et al.*, 1989; Coyne & Orr, 1997; Grant & Grant, 1997), that premating isolation, through behaviour more than morphology, is stronger and evolves more rapidly than postzygotic isolation (hybrid inviability or sterility). This generalisation probably applies to honey bees also. For the two most closely related species pairs of honey bees (*A. nigrocincta/A. cerana* in Sulawesi; *A. nuluensis/A. cerana* in Borneo), behavioural isolation by timing of mating flights would prevent almost all contact between reproductives of the two species. Morphological differences in male genital structure are minor (Otis & Hadisoesilo, 1999; G. Koeniger, pers. comm.) and insufficient to prevent interspecific mating. Experimental crosses between these pairs of species have not yet been performed, but they are so similar genetically that hybrids are likely to be viable.

With mating flights of different *Apis* species being almost completely separated in time, it is difficult to reconstruct past processes that caused the observed patterns. However, known aspects of the mating system of honey bees may provide some clues. The non-overlapping mating flight distributions depicted in Figure 1, particularly between the closely-related species pairs mentioned above, are suggestive of reproductive character displacement (RCD) through reinforcement (the selection for premating isolation as a result of the reduced fitness of hybrids). There is no empirical evidence of natural hybridisation that must occur for reinforcement to operate. However, *A. mellifera* queens do mate interspecifically with *A. cerana* drones on Tsushima Island, Japan, when forced into sympatry; no hybrid adults were produced (T. Yoshida, unpubl. obs.). The reinforcement hypothesis cannot account for the non-overlapping drone flight distributions of species that could not physically mate because of size and genital differences (e.g., *A. dorsata* and the dwarf honey beee; *A. andreniformis* and *A. cerana*; *A. florea* and *A. cerana* in Sri Lanka).

We offer an alternative explanation: reproductive interference. Drones of most (probably all) species orient towards and are attracted to 9-ODA (Free, 1987), a shared component of queen mandibular pheromones (Plettner *et al.*, 1997). It has been observed that when placed in sympatry in Europe, *A. cerana* queens failed to mate, presumably because of interference from the larger numbers of *A. mellifera* drones. Successful matings of *A. cerana* queens occurred only when colonies were isolated (Ruttner & Maul, 1983). Of the three Asian honey bee species recognized at the time, Free (1987, p. 99) wrote, "Because of disparity in size and other anatomical differences interspecific mating is unlikely to occur, but interspecific attraction alone could delay and, possibly prevent, natural mating." Studies of closely related allopatric species of honey bees forced into sympatry are necessary to distinguish between reproductive character displacement through reinforcement as compared to the reproductive interference hypothesis.

Apis florea and *A. cerana* in Thailand are completely isolated despite the overlap in their drone flight distributions. The RIMs responsible for this warrant further study, particularly of the relative attractiveness of their very different queen pheromone blends (Plettner *et al.*, 1997) to drones.

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REFERENCES

COYNE, J A; ORR, H A (1997) "Patterns of speciation in *Drosophila*" revisited. Evolution *51*: 295-303.

FREE, J B (1987) Pheromones of social bees. Comstock Publishing Associates; Ithaca, New York; 218 pp.

GAVRILETS, S; BOAKE, C R B (1998) On the evolution of premating isolation after a founder event. American Naturalis*t* 152: 706-716.

GIDDINGS, L V; KANESHIRO, K Y; ANDERSON, W W (eds) Genetics, speciation, and the founder principle. Oxford University Press, New York, USA; pp. 373.

GRANT, P R; GRANT, B R (1997) Genetics and speciation of bird species. Proceedings of the National Academy of Sciences of the USA 94: 7768-7775.

HADISOESILO, S; OTIS, G W (1996) Drone flight times confirm the species status of *Apis nigrocincta* Smith, 1861 to be a species distinct from *Apis cerana* F, 1793, in Sulawesi, Indonesia. Apidologie 27: 361-369.

HALL, J C (1998) Molecular neurogenetics of biological rhythms. Journal of Neurogenetics 12: 115-143.

KOENIGER, N; KOENIGER, G (in press) Reproductive isolation among species of the genus *Apis*. Apidologie.

KOENIGER, G; KOENIGER, N; TINGEK, S (1994) Crossfostered drones of *Apis cerana* (Fabricius, 1793) and *Apis koschevnikovi* (Buttel-Reepen, 1906) fly at their species specific mating times. Insectes Sociaux 41: 73-78.

KOENIGER, N; WIJAYAGUNASEKERA, H N P (1976) Time of drone flight in the three asiatic honeybee species. Journal of Apicultural Research 15: 67-71.

KOENIGER, N; KOENIGER, G; TINGEK, S; MARDAN, M; RINDERER, T E (1988) Reproductive isolation by different time of drone flight between *Apis cerana* Fabricius, 1793 and *Apis vechti* (Maa, 1953). Apidologie 19: 103-106.

KOENIGER, N; KOENIGER, G; GRIES, M; TINGEK, S; KELITU, A (1996) Reproductive isolation of *Apis nluensis* (Tingek, Koeniger and Koeniger 1996) by species specific mating time. Apidologie 25: 249-264.

LIST, R J (1968) *Smithsonian meteorological tables*. Smithsonian Miscellaneous Collections Vol. 114. Smithsonian Institution Press, City of Washington, USA.

OTIS, G W (1991) A review of the diversity of species within *Apis*. *In* Smith, D R (ed) Diversity in the genus *Apis*. Westview Press; Boulder, Colorado, USA; pp. 29-49.

OTIS, G W (1996) Distributions of recently recognized species of honey bees (Hymenoptera: Apidae; *Apis*) in Asia. *Journal of the Kansas Entomological Society* 69 supplement.: 311-333.

OTIS, G W; HADISOESILO, S (1999) Insights into honey bee biology from *Apis nigrocincta* of Indonesia. *In* Connor, L J; Hoopingarner, R (eds) Apiculture in the 21st Century. Wicwas Press, Cheshire, CT, USA; pp. 69-79.

PLETTNER, E; OTIS, G W; WIMALARATNE, P D C; WINSTON, M L; SLESSOR, K N; PANKIW, T; PUNCHIHEWA, P W K (1997) Species-and caste-determined mandibular gland signals in honeybees (*Apis*). Journal of Chemical Ecology 23: 363-377.

RINDERER, T E; OLDROYD, B P; WONGSIRI, S; SYLVESTER, A H; GUZMAN, L.I. DE; POTICHOT, S; SHEPPARD, W S; BUCHMANN, S L (1993) Time of drone flight in four honey bee species in south-eastern Thailand. Journal of Apicultural Research 32: 27-33.

ROWELL, G A; TAYLOR, O R; LOCKE, S (1986) Variation in drone mating times among commercial honey bee stocks. Apidologie 17: 137-158.

RUTTNER, F; MAUL, V. (1983) Experimental analysis of reproductive interspecies isolation of *Apis mellifera* L. and *Apis cerana* Fabr. Apidologie 14: 309-327.

TINGEK, S; KOENIGER, G; KOENIGER, N (1996) Description of a new cavity nesting species of *Apis* (*Apis nluensis* n. sp.) from Sabah, Borneo with notes on its occurrence and reproductive biology. Biologica Senckenbergiana 76: 115-119.

YOSHIDA, T; SAITO, J; KAJIGAYA, N (1994) The mating flight times of native *Apis cerana japonica* Radoszkowski and introduced *Apis mellifera* L in sympatric conditions. Apidologie 25: 353-360.

Figure 1 Comparison of temporal distributions of mating flights by *Apis* species in six locations: Chanthaburi, Thailand (Rinderer *et al.*, 1993); Tenom, Sabah, Malaysia (Koeniger *et al.*, 1996); Anuradhapura, Sri Lanka (Koeniger & Wijayagunasekera, 1976); Batu Pahat, Johor, Malaysia (G.W. Otis, A. Zainal, & M. Mardan, unpubl. obs.); Bontobulaeng, S. Sulawesi, Indonesia (Hadisoesilo & Otis, 1996) and Tabo Tabo, S. Sulawesi (*A. dorsata binghami*, G.W. Otis & S. Hadisoesilo, unpubl. obs.); and Tokyo, Japan (Yoshida *et al.*, 1994). Key to species: *A. andreniformis*, vertical lines; *A. florea*, dots; *A. cerana*, crossed diagonal lines; *A. dorsata*, dark stippling; *A. nuluensis*, solid gray fill; *A. koschevnikovi*, bold diagonal lines; *A. nigrocincta*, horizontal dashes. The time of sunset in each location is indicated by a 12-pointed star.