Abstract Male *Hybomitra illota* (Osten Sacken) were found aggregating in clearings in wooded areas in Rondeau Provincial Park, Ontario, Canada. At these sites they perched on a variety of substrates, and made frequent flights in pursuit of insects flying overhead. We know that these pursuit flights were part of the mating behavior because some pursuits of *Hy. illota* resulted in copulation. We call the aggregation sites "mating arenas" and the behavior exhibited by males "perch-and-pursue". Aggregation occurred only on sunny days, when ambient temperatures exceeded 18°C. Males perched in sunny areas, except during hot afternoons, when some males were found in dappled shade. Some marked males remained at or returned to sites for up to 13 d, but most males did not remain at the same area within a site, even during the same day.

The contents of the esophageal diverticula of males were depleted daily. Concentration of diverticular carbohydrates changed through the season. Thoracic temperatures of males were high (*ca*. 37°C) and were regulated, probably both behaviorally and physiologically.

The sites and behavior of male *Hy. illota* at aggregation arenas bear some resemblance to lek sites and lekking in vertebrates. Males are aggregated in an arena, but, within the perching component of the behavior, we saw no evidence of male territoriality, display, or female choice. However, competition, display, or mate choice could occur within the pursuit-flight component.

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Introduction

Male Tabanidae engage in a variety of activities as a prelude to mating. These behaviors have been observed for nearly 100 species (Wilkerson *et al.* 1985), and include hovering (either continuously or intermittently) (Magnarelli 1985) and 'waiting' (Catts and Olkowski 1972; Leprince *et al.* 1983). Detailed studies of these behaviors are rare and, as a result, our ability to relate the behaviors to reproductive strategies in the family and to mating systems in general is severely hampered.

Male *Hybomitra illota* (Osten Sacken) are known to aggregate at the edges of bodies of water and in clearings (Pechuman 1981) but little else is known about this activity. As part of a study investigating the aggregation and mating behaviors of several species of Tabanidae (Taylor 1987), we examined 4 aspects of the biology of aggregating male *Hy. illota*: (1) the timing of aggregation in relation to environmental variables; (2) the movement of individuals within and between sites; (3) the thoracic temperatures of individuals in a variety of environmental conditions; and (4) daily and seasonal changes in physiological states.

Materials and Methods

General observations. All investigations were conducted between mid-May and early July of 1985 and 1986, at Rondeau Provincial Park, Ontario (RPP) (lat. 42°10' N, long. 81°90' W; UTM: 17 MS 28). RPP is a cuspate sandspit jutting into Lake Erie from the Ontario side. The park contains a large (*ca.* 1100 ha) remnant Carolinian forest and extensive (*ca.* 750 ha) freshwater marshes. It is *ca.* 3 m above lake level and contains many semi-permanent seasonal sloughs that are good breeding locations for insects with aquatic larvae. A herd of *ca.* 400 (A. Woodliffe, Ontario Ministry of Natural Resources, personal communication) white-tailed deer (Cervidae; *Odocoileus virginianus* Zimmerman), that may act as hosts for female *Hy. illota*, was present in the park during the study period.

Because we knew only that male *Hy. illota* could be found aggregating on roadways through forests, and virtually nothing else about the timing and location of aggregation, we initially searched throughout RPP for aggregating male *Hy. illota* through a range of environmental conditions (sunny, cloudy, rainy, cold and warm days), times of day (from sunrise to sunset) and from prior to, to after the seasonal flight period of the flies (early May to mid-July, as measured by Malaise traps). When males were observed aggregating, we noted the location, time and air temperature (at *ca.* 10 cm above ground in the sun), and watched the flies to determine the nature of their activity. Males were found to aggregate in clearings surrounded by vegetation or other visual barriers — an example being a 20×30-m clearing within a woods. Throughout the paper we call these clearings 'sites'.

2 avenues of investigation were pursued throughout the flight season: observations and marking to study the behavior of males (objectives 1 and 2 above); and collections and dissections of males to determine thoracic temperatures and physiological states (objectives 3 and 4). Marking was conducted at a single site (different in 1985 and 1986); we did not conduct destructive sampling at the marking site.

Marking. Males were individually marked in 1985 and 1986 to gain an understanding of how they moved within and between sites, and the activity patterns of individuals at the site. Perched males were netted, carefully removed from the net with one hand and held between the thumb and the forefinger. Marks were placed on the dorsal surface of the thorax using a water-based, indelible ink (Metron Marker Co., California; these inks have been shown to be long-lasting and non-toxic to other insects (Moon 1985)). 2 dots were placed on the thorax, in 2 of 5 positions (each corner and the center). The 10 position combinations with 7 colors allowed 49 color pairs and 490 individual marks. In 1985 a paintbrush was used to apply ink dots to the thorax; in 1986 we used markers with fine (0.5-mm) needles through which regulated drops of ink could be squeezed. Surface tension between the dot of ink and the cuticle was broken, as early trials indicated that dots could flake off. We ensured the dots were dry and the fly was released in a shaded area.

In 1985 males were marked and reobserved irregularly on the same days. In 1986 marking and re-observations were carried out in a regular manner. One day was devoted to marking, and on the next day on which aggregation took place we conducted 30-min censuses of the site, counting and noting (to the nearest 3 m) the location of marked and unmarked males.

Temperature measurements and physiological states. For thoracic-temperature measurements and physiological-state variables, we sampled aggregating males from 4 sites. In 1985 we collected samples of about 5 males, 2 or 3 times in a day, throughout the season. In 1986 sampling was more intense, but less frequent. On 3 d we collected 20 individuals in each of 3 2-h periods: <1000; 1001–1200; and >1200 EST. On 2 d we collected from a different site in each time period; on the 3rd day there were few individuals present at most sites so we collected exclusively from one site.

Males were collected by quickly placing an insect net over them while they perched. Thoracic temperatures were taken using a YSI series-500 hypodermic-thermistor probe (22-gauge, 0.2-s time constant). The probe was kept shaded at ambient temperature to avoid heating the fly when the probe was inserted, and to determine ambient-air temperature immediately after a thoracic-temperature measurement. Males were held within the net's mesh without being touched, to avoid transfer of heat between the fly and the collector's hand. Flies were stabbed in the center of the thorax, usually within 5 s and not more than 10 s of capture. In 1985 the temperature of the probe was read quickly (<1 s) after stabbing, using a hand-held electronic recorder; in 1986 a Hewlett-Packard data-acquisition unit (model 3421) was used, so that temperature data were obtained instantaneously.

All ambient temperatures not otherwise qualified in the text are air temperatures in a sunny area, measured with a shaded thermistor probe. Ambient temperature is used as a composite-variable surrogate for the thermal fluxes that a male experiences at the aggregation site. Although many individual variables might contribute to a male's thermal status, we have not explicitly explored them.

All males collected were placed into a Plexiglass cage (2.5×2.5×16 cm, with 2 screened sides) to be held for dissection. Those not dissected immediately after collection were placed (without access to water) in a cooler with ice, or a refrigerator at 4°C, within 30 min of collecting. Individuals not dissected within 4 h were discarded.

Dissections were done using watchmaker's forceps under a stereoscopic microscope, usually at ×60. Individuals were immersed in a physiological saline (Hagedorn *et al.* 1977) containing a drop of liquid detergent. The ventral surface of the abdomen was torn open near the thorax, and down the side. The esophageal diverticulum ('crop') was grasped at the point where it joins the esophagus, and pulled out intact. Its surface was dried by blotting lightly with bibulous paper. 3 variables were assessed for each specimen: crop volume; refractive index of crop contents; and the amount of fat body.

Crop volume was visually assessed and assigned to an open-ended series of ordinal categories as follows: 0 (nothing visible); 0–0.5 µl (some liquid); and further categories that increased from 0.5 µl by 1-µl increments. These visual assessments were tested periodically by measuring actual crop volumes using microcapillary tubes.

The refractive index of crop contents was measured using a temperature-compensated refractometer (Reichert, Model 10423). This index can be used as a relative measure of the combined concentration of the sugars sucrose, fructose and glucose in liquids (Inouye *et al.* 1980). Crops containing >1.5 µl of liquid were assessed as follows. The blotted crop was pierced with a 2-µl microcapillary tube and *ca.* 1 µl was drawn into the tube by capillary action. The fraction of the tube containing crop fluid was recorded. The tube was then filled with distilled water and the refractive index of the resulting solution was measured and converted to a refractive index of undiluted crop fluids.

The amount of fat body was visually assessed and assigned to 1 of 4 categories: 0 = no fat, 1 = some fat, 2 = much fat, and 3 = obese.

Physiological-state data were analyzed using multidimensional contingency tables. Log-linear models were fitted using both forward- and backward-selection techniques (Fienberg 1980). The selection technique varied depending on the model being fitted, and knowledge of the biology of the fly. Variables were collapsed into fewer states to increase expected frequencies in cells of sparse tables; collapsing was done by considering the biological significance of categories. The resulting models are not necessarily the only statistically valid ones; they were arrived at through a combination of statistical probabilities and biological knowledge, and are the models of the greatest biological interest to us. The maximum probability of a type-1 error was set at 1% for each stage of the selection procedure so that the overall table-wise type-1 error rate would not exceed 5%.

Log-linear models, generalized linear models (regression and ANOVA) and locally weighted regressions (LOWESS; Chambers *et al.* 1983) were fitted using the TABLES, MGLH and SERIES procedures in SYSTAT (Wilkinson 1986). SAS (SAS Corporation) was used for analysis of variance (PROC GLM) and variance-component estimation (PROC VARCOMP: method MIVEQO). SPEAKEASY (SPEAKEASY Corporation) was used to compute means, variances, standard errors, Student's *t*-tests for independent means and probabilities of *t*- and *F*-values. All systems use double-precision arithmetic. All means are reported ± 1 SE.

The period ('.') in time is an hour-minute delimiter. <Note: deleted here except in the copies of the figures. In the text, I've edited all times to be 4-digit integers, implying hours and minutes instead of decimal hours as is implied by the journal's convention of a period as a delimiter.>

Results

Description of sites, behavior of males, and the environment

Male *Hy. illota* were found aggregating in forest clearings that provided an unobstructed view of the sky. Sites included grassy clearings, the edges of sloughs, roadways, manicured lawns surrounded by forest, and forest paths. Their dimensions ranged from *ca.* 2×2 to 30×40 m. During aggregation, numbers of males at sites ranged from 1 to >50 and varied with time of year, time of day and weather conditions.

Within sites, male *Hy. illota* were distributed non-randomly; they were clumped in smaller (*ca.* 2×2 m) locations that, because of sunshine, substrate and (or) vegetation, were suitable for perching. Throughout the paper we term these smaller locations within a site, 'areas'. The boundaries of areas changed when shadows obscured suitable perches. Males perched on a variety of substrates from *ca.* 0–1.0 m above ground. Perches included bare roadway, fallen logs, grass, leaves, the herb layer of forest paths and small bushes. The reflectance of perches ranged from bleached white (notebooks, some fallen logs) to very dark (bare earth, roadway). Males usually perched in sunny areas, but during the afternoon, when ambient temperatures were high, some males perched in areas with dappled shade from overhead bushes or trees.

In 1985, aggregating male *Hy. illota* were observed on $^{29}/_{47}$ observation days between 11 May and 3 July. In 1986, males were observed to aggregate on $^{16}/_{25}$ observation days between 17 May and 24 June. In both years there was a marked association between clear, warm days and aggregation.

The daily onset of male aggregation was related to ambient temperature. On 11 days in 1986 that precise times (within 0.5 h) of the daily onset of aggregation are known, the mean shade temperature was 18.6 ± 0.35°C. If the morning was cool, the onset of aggregation occurred as late as 1140 (6.8 h after sunrise) and at temperatures as low as 17°C; on warmer mornings, the onset of aggregation was as early as 0755 (3 h after sunrise) and at ambient temperatures up to 20°C (Fig. 1). The onset of aggregation usually coincided with the end of a steep rise in ambient temperature that occurred between 0700 and 0900.
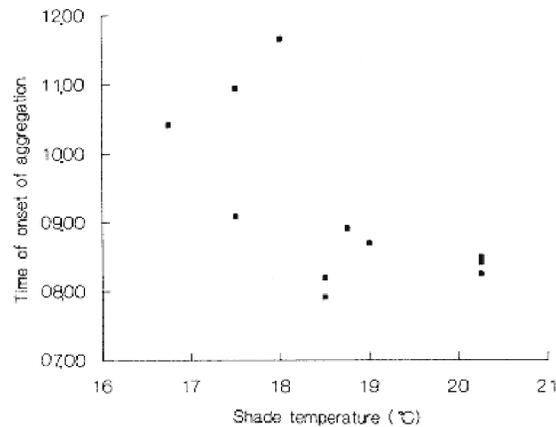

Fig. 1. Time of onset of aggregation *versus* ambient temperature for male *Hybomitra illota* in Rondeau Provincial Park.

Aggregation behavior continued throughout the day if the sun was not obscured by clouds and the air remained warm. Males did not aggregate on cloudy or rainy days, regardless of temperature. On sunny days when air temperature remained below *ca.* 18°C, males did not aggregate, or were scarce. Males were observed aggregating as late as 1558 (4 h before sunset).

Temperatures at the onset of aggregation were always the minimum experienced by aggregating males during a day; the maximum temperatures experienced occurred during the mid-afternoon. In general, the number of males observed was lower in the afternoon than in the morning; the period of the day with fewer males coincides with the period of higher afternoon temperatures. The numbers of males censused at one site and the air temperatures for 2 d in 1986 are shown in Fig. 2. On the warmer day (Fig. 2a), activity began earlier, peaked in mid-morning and declined after ambient temperatures peaked. On the cooler day (Fig. 2b), activity began later and peaked in mid-afternoon, the usual period of low activity. Aggregation ceased at approximately the same time. The maximum number of males observed in one census period was similar on both days.
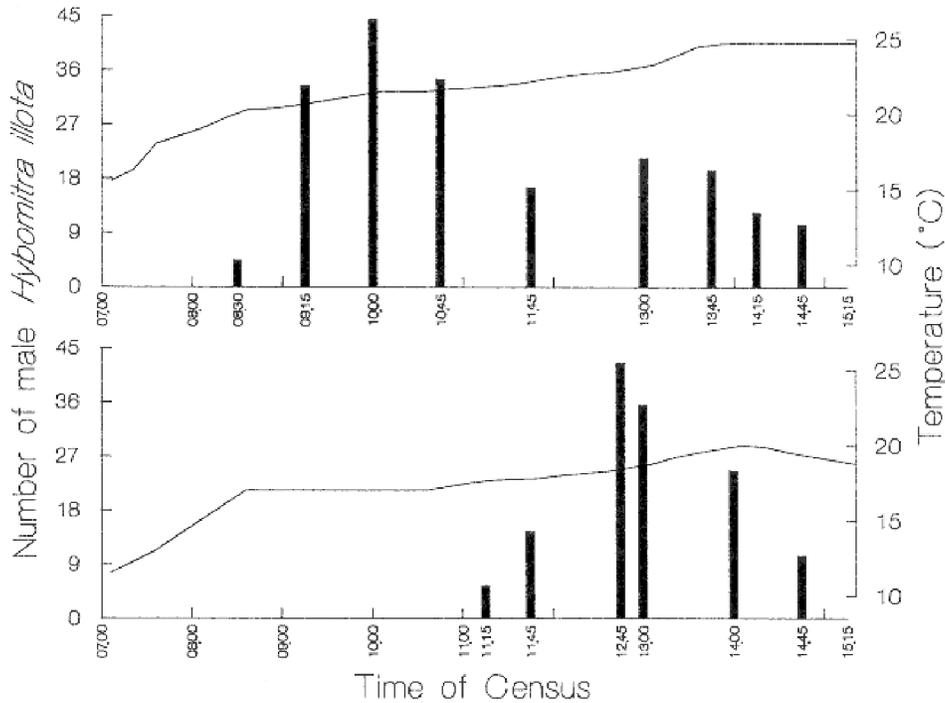

Fig. 2. Numbers of aggregating mate *Hybomitra illota versus* time, and the mean ambient temperature on (a) a warm day and (b) a cool day.

Males at a site exhibited what we term an 'inattentive' or an 'attentive' state. In an inattentive state, male *Hy. illota* did not readily respond to flying objects. They groomed their eyes, legs and wings, and moved about slowly, occasionally probing the substrate with their proboscis if the substrate was damp. When the sun was obscured by clouds, males usually entered an inattentive state until the sun again became visible. Occasionally, males entered an inattentive state while other males were still attentive. The reasons males entered an inattentive state on these occasions are unknown.

In an attentive state, mate *Hy. illota* sat immobile (although some body movements such as abdomen pumping or brief grooming bouts were noted) on substrates and were alert to movements near them. Some objects flying just overhead elicited a pursuit response. These objects included other male and female *Hy. illota*, and other insects. It was impossible to see details of pursuit flights because they occurred with such speed, but general patterns could be inferred by the known outcomes (i.e. copulations) of some flights. Some flights may have occurred spontaneously. During short gusts of wind, males crouched low to the substrate. Under most conditions, however, males usually maintained a higher stance, with the body raised above the substrate.

Males in an attentive state positioned themselves on substrates in predictable ways. In most cases they oriented along the longer axis of a site, facing into an open, sunny area. They did not orient to wind or sun. These conclusions are substantiated by observations of males aggregating on roads. On north-south roads that created a visible path through the forest and forest-canopy, males faced either north or south. On east-west roads, perpendicular, and immediately adjacent to the north-south roads, males faced either east or west. The important factor in their orientation was the orientation of the site itself, not the relative position of the sun or direction of the wind.

Pursuit flights after other Diptera (Sarcophagidae, Syrphidae and other Tabanidae), Hymenoptera and Odonata were observed, in addition to pursuit flights after male and female *Hy. illota*. Pursuits of other insects often resulted in several male *Hy. illota* pursuing each other in a joint flight. Joint flights sometimes involved physical contact between males, such as grappling in mid-air, and tumbling on the ground. Physical contact with species other than *Hy. illota* was never observed. 20 times (over 2 summers), marked, attentive males were observed for periods up to 30 min and the number of pursuit flights recorded. In 189 min of observations these males made 368 pursuit flights (1.95 flights/min; $n = 20$ counts). Flight durations of 5 males were also recorded and ranged from 1 to 16 s (mean 2.8 ± 2.3 s; $n = 88$ flights), so we estimate (crudely) that males spent 9% (2.8 s/flight $\times$ 0.032 flights/s) of their aggregation time in flight. Marked males that were reobserved after a pursuit flight returned to the starting point (mean distance from starting point 7 ± 0.7 cm; $n = 101$ flights).

On 24 occasions we observed pursuit flights that resulted in copulation with female *Hy. illota*. Pairs joined in the air, flew a short distance and landed in grass or on a nearby tree or bush. On the few occasions when the position of each sex was observed, males were flying, and females hung suspended beneath them. Most copulations were observed between 0900 and 1100; on 3 occasions they were timed and lasted 2–5 min. While in copula, other male or female *Hy. illota* did not interact with the pair. Copulations were difficult to observe as they occurred quickly, and pairs disappeared quickly. Males were often observed pursuing female *Hy. illota* that were host-seeking about us as we conducted observations; copulations with these females were not observed.

Stationary males ignored other males that were beside them on the substrate. Occasionally, males landed on top of one another, with no reaction from either individual except to crawl off. We often observed clusters of up to 10 males perched on the same leaf, apparently ignoring one another. Perching sites of marked males were not usurped by other males during pursuit flights.

Movement of males within and between sites

In total, 86 male *Hy. illota* were individually marked and. released at one site on 6 d between 14 May and 16 June 1985. 36 (42%) of these were reobserved at least once, from the day of marking up to 13 d after marking. They were last observed a mean of 3.2 ± 0.5 d after marking. 28 marked males (33%) were reobserved on at least one day subsequent to the marking day. In total, 186 male *Hy. illota* were individually marked and released at one site on 4 d between 14 May and 8 June 1986. 72 (39%) of these were reobserved at least once, from the day of marking up to 11 d after marking. They were last observed a mean of 1.3 ± 0.24 d after marking. 48 marked males (26%) were reobserved on at least one day subsequent to the marking day. The proportion of marked males ever reobserved was similar in both years (42% *versus* 39%; $G = 0.24$, $P = 0.9$). The mean number of days after marking that males were reobserved is not directly comparable between years because of different locations and sampling strategies.

In 1985 28 males were observed on more than two occasions after marking (we ignore the first observation after marking because it is the most likely to have been influenced by the marking process). 5 of these individuals were always observed in the same area within a site (but as there were only 2 observations of each of these males there was little opportunity to observe changes of area). In 148 observations of the 23 males that moved within a site, 67 changes of area were recorded; 31 changes of area occurred between days and 36 within a day.

In 1986 36 individuals were observed on more than 2 occasions after marking. 11 individuals were always observed in the same area within a site (6 were observed twice, and 5 were observed 3, 4, 4, 6 and 19 times). In 183 observations of the 25 males that moved within a site, 54 changes of area were recorded; 23 moves occurred between days and 31 within a day. Marked males seen at least once at a site on a given day were seen on about one-third of the census periods (2 d for which sufficient data are available: mean 2.8 ± 0.3 observations in ten census periods; mean 5.8 ± 1.1 observations in 17 census periods). The farthest distance from the marking site that males were reobserved was *ca.* 750 m.

Thermal characteristics of male Hy. illota

Thoracic-temperature data were collected on 6 d in 1985 and on 4 d in 1986. In 1985 2 individuals with thoracic temperatures $\ll 30°C$ were deemed to be outliers and were discarded; they may have only recently arrived at the site and were not yet warm, or they may have cooled due to delays in handling. To determine if data for all days could be combined, the slopes of the daily regressions of thoracic temperature on ambient temperature were tested for homogeneity. (All days in 1985 were treated as a single sample in this analysis because measurements were from cohorts of 5 individuals collected at a single ambient temperature.) No differences among slopes were found ($P \geq 0.5$), so all measurements were combined.

Thoracic temperatures ranged from 30.5 to 40.8°C (Fig. 3) with a mean of $36.6 \pm 0.17°C$, $8.7 \pm 0.19°C$ above the mean ambient temperature of $27.9 \pm 0.27°C$. Ambient temperatures were significantly more variable than thoracic temperatures ($s^2_{ambient} = 12.9$; $s^2_{thorax} = 5.1$; $F_{181,181(2)} = 2.5$; $P \ll 0.001$). The greatest difference between thoracic and ambient temperature, (Δ_t), was 15.1°C, the least difference, 2.3°C. Δ_t decreased with increasing ambient temperature.
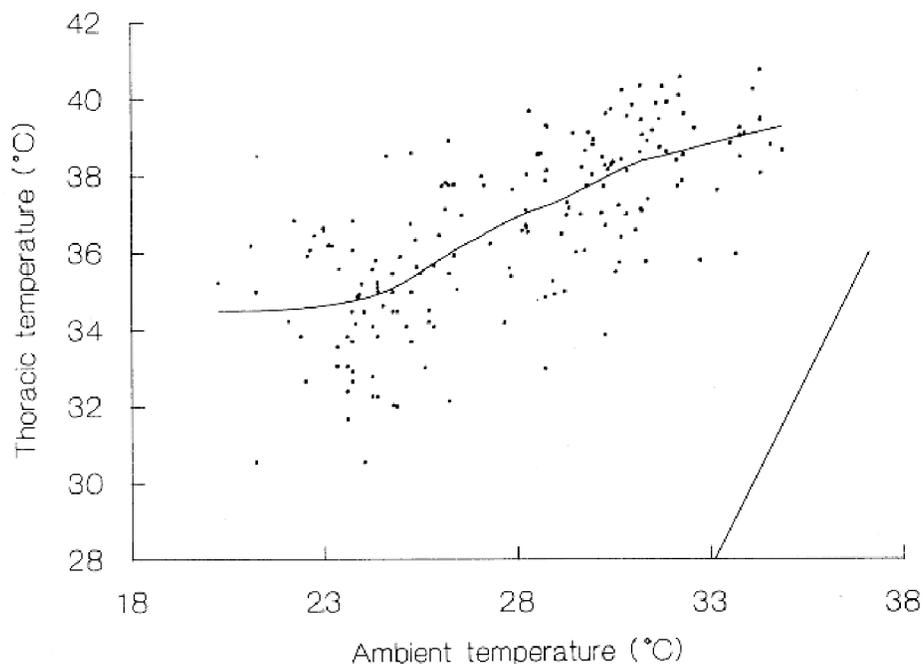

Fig. 3. Thoracic temperatures of aggregating male *Hybomitra illota versus* ambient temperature. The line through the points is a LOWESS curve (locally weighted regression, Chambers *et al.* 1983) to show the trend in the data. The straight-line segment has slope 2, the rate at which thoracic temperature rises in a dead male exposed to the sun (see text); the location of the intercept is arbitrary.

A locally weighted regression (LOWESS; Chambers *et al.* 1983) of thoracic on ambient temperature (Fig. 3) shows a slight leveling of the thoracic temperatures at the highest ambient temperatures and, at the lowest ambient temperatures, a poorly defined curve. The latter is due to the small number of data points in that region and should be ignored. Over most of the range of ambient temperatures, a linear relationship is appropriate.

A linear regression model, thorax $= 24.1 + 0.45 \times$ ambient ($P < 0.0001$) explained 51% of the variance in the thoracic temperatures. One data point (from 16 June) was eliminated from all further analysis as it was an extreme outlier (Studentized residual $= -4.2$, $P < 0.0001$). The slope of the model is <1 ($t = 16.9$; $P < 0.001$), indicating that thoracic temperatures increased more slowly than ambient temperatures. A small, but significant (H_0: slope $= 0$; $F = 5.3$, $P = 0.02$), decrease in residual variance with increasing predicted thoracic temperature suggests a stabilization of thoracic temperatures at higher ambient temperatures.

The slower increase in thoracic relative to ambient temperature, coupled with the significantly lower variance in thoracic temperatures at higher ambient temperatures and the indication in the LOWESS plot of a stabilization of thoracic temperatures at high ambient temperatures, all suggest that males regulate their thoracic temperatures to some extent.

Ambient temperatures in the shade and sun, and thoracic temperature of dead males in the sun were measured at 5-min intervals throughout one day in 1986. In addition the maximum thoracic temperatures of live males held in the sun for 3–4-min periods, next to substrates that males had used as perching sites, were collected at 7 times during the same day to provide an estimate of the maximum possible passive thoracic temperatures of males (Fig. 4).
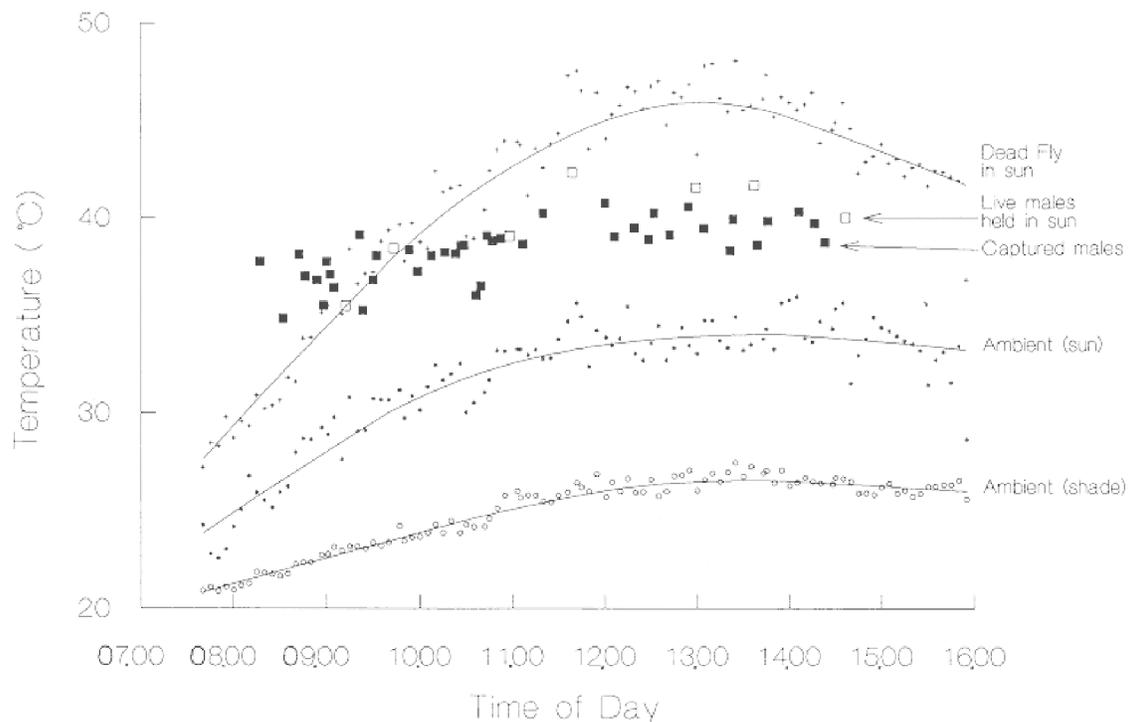

Fig. 4. Ambient temperatures in sun and shade, thoracic temperature of a dead *Hybomitra illota* male in the sun, thoracic temperatures of living males of *Hy. illota* held in the sun, and thoracic temperatures of aggregating males of *Hy.illota*, *versus* time of day (a single day in 1986). Solid lines are LOWESS curves.

All ambient temperatures increased until *ca.* 1130 when ambient temperatures in the sun and shade stabilized. Thoracic temperatures of live males held in the sun increased to 42.5°C at 1130, and then remained stable near 41°C for the rest of the measurement period. Until 1130 thoracic temperatures of live males held in the sun rose in parallel with the thoracic temperature of the dead male; the dead-male temperature continued to rise until *ca.* 1300. The thoracic temperature of the dead male rose as a linear function of ambient temperature, but at twice the rate (linear regression; dead fly $= 1.97 \times$ sun $- 21$; $P < 0.001$). Thus, if live, aggregating males were warming passively by basking, the slope of thoracic *versus* ambient temperatures would be near 2.

Thoracic temperatures of aggregating males were significantly more variable before 1130 than after ($F_{26,14\,(2)} = 3.1$; $P = 0.03$), and stabilized in the hotter part of the afternoon. This may be partly explained by considering that males that have just entered the mating site may take time to warm up. If sampled during this warm-up period, they will, as a group, have lower thoracic temperatures, and show greater variance. Also, at high ambient temperatures, more stringent regulation of temperature may be necessary to avoid overheating, thus reducing the variance in thoracic temperatures of males during that time.

Physiological characteristics of males

The relationships between the 4 variables: CROP-SIZE, FAT-BODY, TIME-OF-DAY and TIME-OF-SEASON were examined using log-linear models. Data from 306 males collected in both years were included in the analysis. The original classes of the 4 variables were collapsed into smaller subsets by inspecting the data and judging meaningful subsets *a priori*. These data are summarized in Table 1. We assumed no differences among sites.

Table 1. Percentage occurrence of physiological-state variables for male *Hybomitra illota* in Rondeau Provincial Park: (A) CROP-SIZE as a function of TIME-OF-DAY; (B) CROP-SIZE as a function Of TIME-OF-SEASON; (C) FAT-BODY as a function Of TIME-OF-DAY; and (D) FAT-BODY as a function of TIME-OF-SEASON.

		Time of day (hours)				Time of season	
		08.00 −10.00	10.01 −12.00	12.01 −16.00		14 May −7 June	8 June −24 June
Crop size (µl)							
0−0.5	(A)	29	32	60	(B)	49	33
−1.5		26	32	33		31	30
−2.5		26	27	5		15	22
>2.5		17	8	3		5	12
Fat body							
Little	(C)	54	76	82	(D)	63	84
Lots		46	24	18		37	16
n		82	105	119		178	128

TIME-OF-SEASON was divided into an early and a late season (split at 7 June; data for 1985 and 1986 were combined); TIME-OF-DAY was divided into 3 periods, FAT-BODY was divided into 2 categories and CROP-SIZE was divided into 4 categories. These divisions resulted in a 2×3×2×4 table (TIME-OF-SEASON × TIME-OF-DAY × FAT-BODY × CROP-SIZE).

The model containing the following 2-way interactions (and all main effects) provided a good fit: {TIME-OF-SEASON × TIME-OF-DAY}, {CROP-SIZE × TIME-OF-DAY}, {FAT-BODY × TIME-OF-SEASON} and {FAT-BODY × TIME-OF-DAY}.

The {TIME-OF-SEASON × TIME-OF-DAY} interaction reflects the initial experimental design and simply shows that sampling was not distributed evenly through the day in both parts of the season; this term is otherwise not biologically interesting. The interaction {CROP-SIZE × TIME-OF-DAY} shows that crop sizes diminish through the day (Table 1). There are 3 possible explanations: (a) individuals metabolize crop-sugars directly and rapidly; (b) individuals convert crop-sugars to fats as the day progresses; (c) different subsets of the population are being sampled through the day. The {FAT-BODY × TIME-OF-SEASON} interaction shows a decrease in fat body from early to later in the year; the decrease could be a result of population aging, with progressive depletion of larval fat-body reserves, and (or) the late-season emergence of adults with reduced fat reserves.

The interaction between FAT-BODY and TIME-OF-DAY was unexpected and prompted a further look at the data. A pattern of decreasing fat through the day was most evident on a day in 1986 when samples were taken from 3 sites at different times of the day. To test the hypothesis that CROP-SIZE or FAT-BODY differed among sites, we examined the two 4-way tables containing CROP-SIZE or FAT-BODY with the 3 variables TIME-OF-DAY, TIME-OF-SEASON and LOCATION. The resulting models indicated that the effects of location within RPP are likely an important physiological variable. There may be differences in larval habitats on a micro-scale, differences in larval feeding habits, or differences in adult energy utilization or emergence patterns, that cause some males to have different amounts of fat-body at the same time of year.

The refractive indices of crop contents are a relative measure of carbohydrate reserves. We selected 2 d from 1985 and 4 d from 1986 for analysis ($n = 94$; all days had $n \geq 9$). A 3-way, random-effects ANOVA was used to test the null hypothesis that DATE, CROP-SIZE and FAT-BODY have no effect on the concentration of carbohydrates in the crops of aggregating males. Refractive indices were arcsine-square-root transformed to reduce non-normality of the data. Variance components for all effects were estimated. All main effects and interactions were clearly non-significant ($P > 0.1$) except for DATE ($P < 0.005$). The large variance component due to DATE (37%) indicates that sugar sources or concentrations change through the season. This hypothesis is further substantiated by comparing the mean refractive index of crop contents on 3 and 4 June 1986 ($63.1 \pm 1.6\%$, $n = 44$) with 14 and 16 June 1986 ($45.0 \pm 2.0\%$, $n = 27$). Either the males make use of different carbohydrate sources as the season progresses, or the carbohydrate sources themselves vary in sugar concentration through time.

Discussion

Aggregation behavior in male *Hy. illota* has been reported twice in the literature. Philip (1931) observed aggregating males in a clearing near the shore of a marsh in 1925. He did not associate the behavior with mating. Pechuman (1981) observed many males along the shore of a lake in New York State and found mating pairs. In the present study we conclusively link the aggregation behavior of male *Hy. illota* with mating. We term the mating strategy used by *Hy. illota* 'perch-and-pursue'. The term 'waiting', which has previously been used to describe this behavior (Catts and Olkowski 1972; Leprince *et al.* 1983), does not fully summarize all the components.

The Hy. illota mating arena and mating system

The mating systems of some insects are sometimes compared to vertebrate leks (e.g. Thornhill and Alcock 1983). A lek is 'a type of territory held by males of a certain species, and used solely as a communal mating ground' (McFarland 1987). At the lek, males aggregate, interact, compete with one another, and display to females. Females at a lek may select a mate.

Mating in *Hy. illota* occurs at sites where males aggregate; we call these sites 'mating arenas'. These arenas have similarities to leks. However, we use the more neutral term 'arena' because we cannot confirm the presence of essential elements of lek behavior, nor do we know if mating occurs at other times or locations. Within an arena, male *Hy. illota* are attentive, and pursue female *Hy. illota* flying overhead and, at least on some occasions, these females are receptive to mating.

Within the perching component of the aggregation behavior of male *Hy. illota*, we do not see all the components of behavior typical of a lek. Perched males do show highly clumped dispersions within the mating arena and some male *Hy. illota* return to or remain at the same mating arena for up to 2 wk. However, movements within a site occur daily and our observations of marked males show that if males are defending territories, individuals are abandoning and re-establishing them exceedingly frequently during the day. We observed no interaction or aggression among the perched males, no male display or courtship behavior, and no females inspecting males.

However, within the pursuit-flight component of the aggregation behavior of male *Hy. illota*, we do observe some features that may be characteristic of a lek-type mating system. Males are aggregated, they often pursue females as a group, and they frequently interact with one another. These encounters are easily interpreted as investigative; a male *Hy. illota* flying overhead at great speed must look much like a female. Males may pursue such targets to determine if they are female *Hy. illota*. Perching males could use males in pursuit as a clue to the presence of a female, resulting in a group of males pursuing each other or jointly pursuing a female. When a group of males pursues a female, short, subtle displays could occur within moments of copulation. Males could dominate other males during the pursuit flight, and thus force males into other parts of the arena. Female choice may be exhibited within, or as a consequence of the pursuit flight, which in itself could be a display. Sophisticated analyses of the details of the pursuit flight and behavior of both sexes before the moment of copulation could change our perception of the importance of these events.

Behavior and the mating arena

Male *Hy. illota* orient themselves with respect to the long axis of a mating arena; males of several other species of Tabanidae also orient themselves within mating arenas in this way (Taylor and Smith, unpublished observations). Most other workers report that male Tabanidae orient themselves into the wind or toward the sun (e.g. *Tabanus bishoppi* Stone: Blickle 1955; *Hybomitra lasiophthalma* (Macquart): Magnarelli 1985). We suspect that previous studies may have neglected to consider that the configuration of the mating arena itself may be an orientation cue. Interestingly, Leprince *et al.* (1983) found males of several species of Tabanidae hovering parallel to a path on calm days, and into the wind on windy days.

Vision is important in the Tabanidae (Allan *et al.* 1987) and our observations suggest that vision plays an important role in mating. The swarming behavior of *Bibio marci* (L.) (Diptera: Bibionidae) is similar to that of some Tabanidae (*see* Zeil 1983). The enlarged upper ommatidia in male *B. marci* are used for tracking targets moving overhead; the female-typed ventral ommatidia are used for orientation. Target images are kept centered on the midline, where limited binocular vision occurs (Zeil 1983). In many male Diptera, including male Tabanidae, the dorsal ommatidia are particularly sensitive to light in the ultraviolet (UV) or near-UV range and the female and ventral male ommatidia are less UV-sensitive (Burkhart and de la Motte 1972; Allan *et al.* 1987). The morphology of the eye (broad in front and above, slender at the sides) suggests that male Tabanidae have better frontal than peripheral vision.

The orientation of male *Hy. illota* at the mating arena and the morphology of their eyes are linked. The mating arenas of *Hy. illota* provide unobstructed overhead views; a female flying over a group of males will contrast strongly with the clear blue/UV sky. Males that are oriented along the long axis of a mating arena will detect females flying along that axis, and will be able to detect them from a greater distance than could males oriented in other ways (frontal *versus* peripheral vision). A male will thus be able to rapidly detect a female flying toward the aggregation, center her on his ocular midline (to provide optimal binocular vision for tracking), track, capture, and copulate with her.

Physiological states of males at the mating arena

70% of males collected in the first 3 h of aggregation had >0.5 μl (often ≥ 0.5 μl) of liquid in the crop whereas 60% of males collected in the last 4 h of aggregation had <0.5 μl. Either males spend most of the day at the aggregation site and rapidly deplete crop contents, or males with little crop contents aggregate at later times of the day than do males with well-filled crops. Our observations of some marked males throughout the day support the former hypothesis. Magnarelli (1985) showed a similar pattern of crop depletion during aggregation in *Hy. lasiophthalma* and *Hy. aurilimba* (Stone). Inasmuch as most Diptera use carbohydrates for flight (Downer 1980), these observations are not unexpected. Because flight is so important to aggregating males, and because crop contents are depleted quickly, frequent (probably daily) acquisition of sugars to finance the behavior must also be important.

The aggregation behavior of *Hy. illota* most closely resembles that of *Hy. microcephala* (Osten Sacken), the males of which sit immobile on shrubs or ground in sunny locations, and pursue other males (Leprince *et al.* 1983). In direct contrast to our samples of male *Hy. illota*, few male *Hy. microcephala* had recently fed on sugars but most had abundant fat body (Leprince *et al.* 1983). The smaller fat body, coupled with the larger crop volumes observed in male *Hy. illota*, could be due to interspecific, regional and (or) seasonal variation in energy-utilization strategies.

Thoracic-temperature regulation of males at the mating arena

The behavior of male *Hy. illota* was only partly temperature dependent; on warmer days, males began aggregating *ca.* 3 h after sunrise but on cooler days the onset of aggregation was delayed.

2 facts suggest that male *Hy. illota* regulate their thoracic temperature: thoracic temperatures of male *Hy. illota* were higher, but less variable than ambient temperatures; and the slope of the regression of thoracic on ambient temperature was significantly <1. The presence of males in the shade during hotter afternoons suggests behavioral regulation, and the high thoracic temperatures and their relative stability at higher ambient (*ca.* 30°C) temperatures suggest physiological regulation.

The ability to regulate thoracic temperature means that the interval during the day when the species can aggregate is expanded; males can stay warmer when the temperature is low, and cooler when it is high. It also means that males can ensure that they are physiologically ready to take flight rapidly and pursue females flying over the mating arena. The dynamics of thoracic temperatures during these flights, and in the warm-up period before males begin aggregation, are unknown.

Flight is clearly an important component of the daily activities of male as well as female Tabanidae, and carbohydrates are essential for flight. Maintaining a high thoracic temperature through a range of ambient temperatures ensures that males maintain a physiological readiness for rapid pursuit flights. A fit male will be one that can make repeated pursuit flights, at high speed, over extended periods.

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