Testing hypotheses on egg number and size in black flies (Diptera: Simuliidae)

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ABSTRACT: Field-collected gravid females were used to test a series of inter- and intraspecific hypotheses on egg size and number in 23 species of black flies. Qualitative differences between bird and mammal blood did not result in significant differences with respect to fecundity properties. The five species with overwintering larvae produced more eggs than did the 18 species with overwintering eggs. Contrary to prediction, egg size was correlated significantly with body size in only two of 17 examined species; indeed, in three species this relationship was negative. However, the association between female size and egg number within species was positive. Strong support was found for a tradeoff between egg size and number across species but not within species. The present study shows similarities with other dipterans but also substantial variability, probably reflecting a high degree of flexibility. The results are primarily discussed in an evolutionary context, but they also provide useful information for black fly management. *Journal of Vector Ecology* **29 (2): 248-256. 2004.**

Keyword Index: Simuliidae, fecundity, Sweden, tradeoffs, stream insects.

INTRODUCTION

Black flies constitute a large and important family (Simuliidae) of the order Diptera. The significance of black flies is manifested in both aquatic (streams/rivers) and terrestrial (riparian/upland) ecosystems and as economically important pests and vectors of disease agents to birds and mammals, including domestic animals and humans (Crosskey 1990, Malmqvist et al. 2004a, Malmqvist et al. 2004b). Insight into the relationships of egg numbers and size as well as tradeoffs between these can provide valuable ecological information. Black flies are suitable for such studies because they belong to a phylogenetically and morphologically homogenous insect family and show diverging life-history strategies with respect to blood-feeding and overwintering. Detailed information on black fly fecundity relationships may also improve the prospects for successful management.

Egg number is related to the fitness of an animal. In obligately autogenous black fly species (those with untoothed mouthparts not capable of acquiring blood), the number of developed eggs in gravid females is synonymous with lifetime fecundity, whereas in anautogenous species (those requiring blood for egg maturation) several cycles of egg development and oviposition are possible, implying that actual fecundity can be considerably less than potential fecundity. Here, we test a number of hypotheses pertaining to egg numbers of black flies. (1) *Ornithophilic and mammalophilic species are similar in fecundity.* Crosskey (1990) suggested that there would be no difference due to host type. However, the quality of blood is different, which potentially could influence reproductive investments. For example, blood sugar concentration is twice as high in birds as in mammals (Baptista and Welty 1988). Globulin protein, the relative number of leucocytes, and blood pressure are also higher in birds, and their red blood cells have a nucleus, which is absent in mammalian red blood cells (Baptista and Welty 1988). Moreover, higher fitness, determined by increased number of matured eggs, was demonstrated in a black fly species (*Prosimulium mixtum* Syme & Davies) fed on bird blood than on blood from humans (Mokry 1980a). (2) *Body size and egg number are related* (Crosskey 1990). Size-fecundity relationships are little studied in anautogenous species, which comprise the majority of black flies (89% of the species in northern Sweden, Adler et al. 1999) and are of greatest economic, and probably ecological, significance. Published findings are inconsistent: Cheke et al. (1982) and Pascuzzo (1976), who examined Afrotropical and Nearctic species, respectively, found a positive relationship between size and fecundity, whereas Bellec & Hebrard (1984) and Mokry (1980b), who also

studied Afro-tropical and Nearctic species, did not. If blood meals are important for egg development, this relationship should be weak, although conditions during larval life probably also have an influence. Most studies on black fly egg numbers have involved obligately autogenous species or primiparously autogenous species (those not needing blood for the production of a first egg batch), and they generally show that larger females have more eggs than do smaller females (Chutter 1970, Pascuzzo 1976, Colbo 1982). Blood-seeking females normally would need to disperse greater distances from their natal rivers to find a host, and this, coupled with the costs of surviving host defensive behaviors, may result in less energy being available for the reproductive effort than in autogenous females. Hence, egg numbers may be relatively low and/or their size small in anautogenous species (Berrigan 1991).

In addition to numbers, the size of eggs is also a fitness component likely to show variation within and across species. We hypothesized that (3) *a tradeoff exists between egg size and number*, and (4) *larger females produce larger eggs than do smaller females of the same species*. Such trends have been observed in many aquatic insects (Statzner et al. 1997), but they have not been tested with black flies. Within insect families, larger species tend to produce larger eggs, suggesting morphological constraints on egg size (Fox and Czesak 2000). Within populations, larger females tend to produce larger eggs, again suggesting morphological constraints on egg size, although the relationship is typically weak and exceptions are numerous (Fox and Czesak 2000). A consequence of such constraints would be a tradeoff between egg size and number (Smith and Fretwell 1974). In anautogenous simuliids, which obtain most resources for egg production from blood feeding, the female's diet, rather than its size, should be the more important determinant of egg size (Fox and Czesak 2000). In obligately autogenous species, and for the first gonotrophic cycle of primiparously autogenous species, however, resources obtained exclusively during the larval stage should govern egg size. Further, prosimuliines typically have larger eggs than many simuliines (Crosskey 1990), suggesting a phylogenetic component.

Statzner et al. (1997) argued that life-history studies might be interpreted more easily if the study organisms are reasonably similar. Black flies are relatively homogeneous in morphology and life style and should be a favorable group for study. Sweden has a rich black fly fauna (Adler et al. 1999), providing an appropriate location for the comparison of life histories. In a project aimed at understanding multiple aspects of the ecology of adult black flies in northern Sweden, extensive collections were made using vehicle-mounted nets. Here we use the gravid females from those collections to test the four hypotheses outlined above.

MATERIALS AND METHODS

A vehicle-mounted net (Davies & Roberts 1980) was used to trap airborne insects along the Vindel and Ume rivers in northern Sweden (latitude: 64-65°N). 175 samples were obtained along the rivers on 34 dates in 1998-2000. Additional data from 2001 derive from 204 trapping samples taken on 19 dates along these rivers, as well as the Skellefte, Pite, Lule, Kalix and Torne rivers (65-67°N). Trapping was performed from early June to mid-September. Samples were collected along road segments that ranged in length between 0.5 and 18 km (average 5.3 km). Trapping was performed unequally over the course of the day, with no trapping between 02.00 and 06.00 h. More than 80% of the catches were taken between 10.00 and 22.00 h; greatest flight activity can be expected in mornings and evenings (Davies and Roberts 1980). Collected flies were placed in 70% ethanol. Representative specimens are housed in the Clemson University Arthropod Collection, Clemson, SC, U.S.A.

In the laboratory, captured black flies were identified to species, and wing length, egg number, and egg size were measured in gravid females (Table 1). Species identification was aided by a provisional key to Swedish black flies modified from one developed for North American species (Adler et al., 2004). Eggs of gravid females were dissected out, teased apart, and enumerated. We analyzed 25 females of each species, which was possible for only 14 taxa, viz. *Cnephia pallipes*, *Metacnephia lyra*, *Simulium annulus, S. curvans, S. intermedium, S. murmanum, S. ornatum, S. reptans, S. rostratum, S. rubtzovi, S. subpusillum, S. transiens, S. truncatum,* and *S. tuberosum*. For three additional species, *Metacnephia saileri, Prosimulium hirtipes,* and *Simulium noelleri*, 10-18 gravid females were obtained, and for six other taxa <10 females were collected and could be included in some analyses (Table 1).

Black fly eggs are eccentrically ovoid, hence their volume is difficult to model. Because egg shape is uniform across species (with few exceptions), we measured the length of the egg as a proxy for size (Crosskey 1990). Measurements were performed on a subsample of five eggs with a compound microscope at 100x magnification.

One wing of each female was removed at its base and placed in a drop of water beneath a cover slip. The wing was measured from its apex to the proximal margin of the basal radial cell under a dissecting microscope at 20x magnification.

Table 1. Means, with standard deviations (sd), of body size (as wing length), egg size (length), and egg numbers in 23 black fly taxa. Information on overwintering strategy (E=as eggs; L=as larvae), host (O=birds; M=mammals; A=autogenous) and voltinism (M=multivoltine; U=univoltine) are also given. Identification to species is not possible for some Swedish species. Thus, '*S. tuberosum*' potentially includes *S. tuberosum* s. s., *S. vulgare* Dorogostaisky, Rubtsov & Vlasenko, and *S. annulitarse* Zetterstedt; '*S. rubtzovi*' potentially includes *S. rubtzovi*, *S. morsitans* Edwards, and *S. curvistylus* Rubtsov; and '*S. truncatum*' potentially includes *S. truncatum* and *S. paramorsitans* Rubtsov. Females of *S. subpusillum* Rubtsov and *S. pusillum* are not readily separated and either or both of these two species might be included under '*S. subpusillum*'. '*Simulium aureum*' refers to *S. aureum* s. s. or *S. angustipes* Edwards. Several closely related species might be included under '*S. vernum*'.

Species	n	Mean Wing Length (mm)	sd	Egg Size (mm)	sd	Egg Number	sd	Over-wintering Strategy	Host	Voltinism
Cnephia pallipes	25	3.23	0.19	0.287	0.015	212	47.1	E	A	U
Metacnephia lyra	26	3.00	0.28	0.281	0.012	163	45.4	E	O	U
M. saileri	18	3.73	0.44	0.289	0.011	212	55.1	E	O	U
Metacnephia sp.	1	2.75	-	0.304	-	156	-	E	O	U?
Prosimulium hirtipes	11	3.83	0.32	0.283	0.067	337	125	L	M	U
Simulium annulus	25	3.15	0.13	0.203	0.009	587	128	L	O	U
S. aureum Fries	1	3.10	-	0.190	-	801	-	E	O	M
S. curvans	25	2.98	0.38	0.223	0.016	295	180	E	O	U
S. dogieli	3	2.98	0.32	0.241	0.046	218	30.7	E	O	U
S. equinum	2	3.13	0.67	0.239	0.038	287	98.3	E	M	M
S. intermedium	26	3.33	0.30	0.241	0.027	379	113	L	M	U
S. murmanum	25	2.89	0.34	0.348	0.014	91	38.5	E	M	U
S. noelleri	10	3.41	0.24	0.246	0.032	254	91.0	E	M	M
S. ornatum	25	3.58	0.24	0.261	0.013	408	110	L	M	M
S. reptans	25	2.50	0.21	0.231	0.009	141	23.7	E	M	M
S. rostratum	25	2.96	0.23	0.224	0.007	271	119.1	E	M	M
S. rubtzovi	25	2.66	0.26	0.220	0.008	201	84.3	E	M	U
S. subpusillum	25	2.51	0.27	0.218	0.016	132	74.5	E	M	M
S. transiens	25	2.33	0.35	0.193	0.011	182	37.5	E	O	M
S. truncatum	29	2.67	0.23	0.300	0.029	147	62.9	E	M	U
S. tuberosum	25	2.45	0.21	0.250	0.016	140	36.4	E	M	M
S. vernum	6	3.09	0.60	0.251	0.021	213	63.1	L	O	M
Stegopterna	6	2.38	0.10	0.336	0.015	46	12.7	E	M	U

Table 2. Comparison of intraspecific winglength (x) vs. egg number (y) for relationships based on at least six observations. ns = non significant; * *P*<0.05; ** *P*<0.01. *** *P*<0.001.

Species	Relationship	r^2	n	Significance	Direction
C. pallipes	y = 218.65x - 494.24	0.746	25	***	+
M. lyra	y = 94.049x - 118.13	0.312	26	**	+
M. saileri	y = 75.31x – 69.34	0.366	18	**	+
P. hirtipes	y = 193.29x - 403.64	0.247	11	ns	+
S. annulus	y = 629.24x - 1393.4	0.380	25	**	+
S. curvans	y = 370.46x - 809.43	0.783	25	***	+
S. intermedium	y = 236.5x - 408.13	0.399	26	***	+
S. murmanum	y = 60.539x - 84.037	0.284	25	**	+
S. noelleri	y = 122.58x - 152.06	0.293	10	ns	+
S. ornatum	y = 313.48x - 713.39	0.486	25	***	+
S. reptans	y = -29.266x + 214.65	0.069	25	ns	-
S. rostratum	y = 99.156x - 22.546	0.037	25	ns	+
S. rubtzovi	y = 178.82x - 274.35	0.311	25	**	+
S. subpusillum	y = 74.597x - 55.268	0.075	25	ns	+
S. transiens	y = -34.272x + 261.49	0.004	25	ns	-
S. truncatum	y = 139.66x - 226.92	0.423	29	*	+
S. tuberosum	y = 78.429x - 52.03	0.203	25	*	+
S. vernum	y = 65.082x + 11.954	0.383	6	ns	+
Stegopterna sp.	y = 80x - 143.67	0.429	6	ns	+

The statistical methods included linear regression, Pearson's product moment correlation, binomial sign test, and one-way ANOVA on log-transformed data. Tradeoffs between egg number and size were estimated by plotting the residuals from body size (as wing length) versus egg size against the residuals from body size versus egg number (Berrigan 1991).

RESULTS

Over 30,000 black flies from at least 31 species, including 23 species with eggs, were captured along the 379 stretches for a combined distance of 2,002 km. About 2,200 (7.3%) of all females contained eggs. Egg number per female ranged between 19 and 836; 78% of the species contained between 100 and 400 eggs (median 213; Table 1). The species with the most eggs were *Simulium annulus* (average 587 eggs/female) and *S. aureum* (801 eggs/female), the latter based on the number of eggs in the only captured gravid female of this species. The species with the least number of eggs was *Stegopterna* sp. with an average of 46 eggs/female. Across all species, egg size (as length) ranged between 0.16 and 0.39 mm. The largest mean sizes of eggs were found in *S. murmanum* (0.35 mm) and *Stegopterna* sp.

(0.34 mm). The eggs of *S. annulus* and *S. transiens* averaged ≤ 0.20 mm. The eggs of the single female of *S. aureum* were also very small (0.19 mm).

The results of the hypothesis testing are as follows, with the parenthetical numbers referring to the hypotheses listed in the introduction.

(1) *Ornithophilic and mammalophilic species have similar fecundities*. On average, ornithophilic species were larger and contained more and larger eggs than mammalophilic species; however, these differences were not statistically significant (F_{number} = 0.45; $F_{eggsize}$ = 1.37; df = 22; *P*>0.1; with rare species removed F_{number} = 0.96; $F_{eggsize}$ = 0.95; df = 16; *P*>0.1; Figure 1). These results are in accordance with the prediction of no difference in fecundity related to host type.

(2) *Body size and egg number are related*. Regressions showed that egg number as a function of body size (wing length) was highly variable intraspecifically and that significant relationships generally were weak (Table 2). In some cases, nonsignificance might have been associated with low statistical power, but in several well-represented species there was no relationship (*S. rostratum, S. reptans, S. subpusillum,* and *S. transiens*). However, 17 out of 19 species indicated a positive relationship (*P*<0.001;
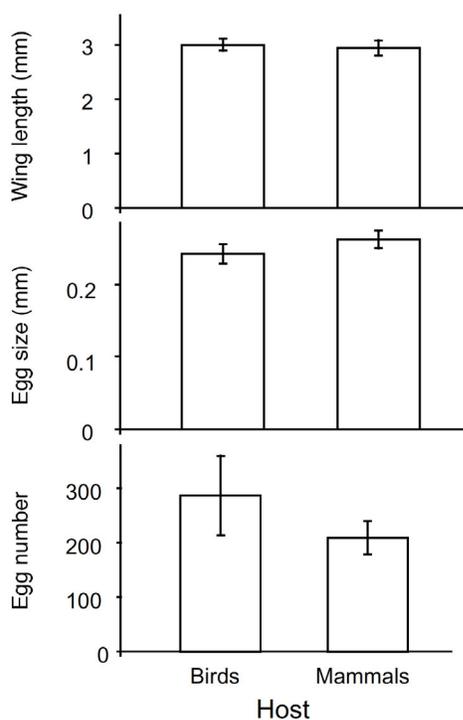
Figure 1. Mean wing length, egg size, and egg number (± SE) in black flies, with respect to host type (birds, n=10; mammals. n=13).

Figure 2. Mean wing length, egg size, and egg number (± SE) in black flies, with respect to overwintering strategy (as eggs or larvae). N_{eggs}=18; N_{larvae}=5.

Binomial sign test). Hence, this hypothesis was verified despite unexplained exceptions and weak patterns within species.

Across species, female size ($F_{1,18}$=8.17; P<0.01) and egg number ($F_{1,18}$=6.09; P<0.05) were significantly greater in species with overwintering larvae (Figure 2). The effect was partly attributed to the highly fecund species, *Simulium annulus*, with an average of 587 eggs/female, which is 2.3 times greater than the overall mean of 255 eggs/female across species. Egg size did not differ with respect to overwintering strategy ($F_{1,18}$=0.09; P>0.05).

(3) *A tradeoff exists between egg size and number*. In intraspecific comparisons, a significant tradeoff between egg size and egg number was found only for *S. annulus* and *S. truncatum,* and in *Cnephia pallipes*, at variance with a tradeoff, egg size and number were positively correlated. In contrast, the interspecific comparisons across 19 species showed highly significant tradeoff relationships (r=0.90; *P*<0.001; Figure 3).

(4) *Larger females produce larger eggs*. This hypothesis was not accepted for any species (n > 17) except *S. intermedium* (*P*<0.001) and *S. subpusillum* (*P*=0.003). *Simulium murmanum*, *S. truncatum*, and *S.*
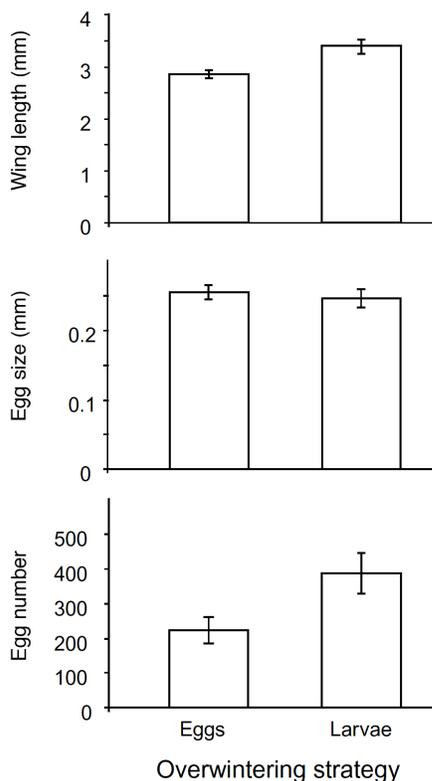
curvans had significant negative correlations (*P*=0.005, 0.002 and 0.0005, respectively). There was no trend between egg size and female size across species (*P*>0.05).

The high variability in the tests stemmed from many sources, including possible errors in measurements, but clearly spatial and temporal heterogeneity were important. For example, in a number of species we saw a relationship between the time of capture during the flight season and egg size. A significant negative trend was present in *Metacnephia lyra, M. salieri, Simulium curvans, S. intermedium, S. ornatum, S. truncatum,* and *S. tuberosum*, whereas the opposite trend was found in *Prosimulium hirtipes*.

DISCUSSION

Egg numbers are correlated with body size in Diptera and tradeoffs exist between egg size and number (Fox and Czesak 2000). Our study demonstrates consistency with this trend for the Simuliidae. However, in species
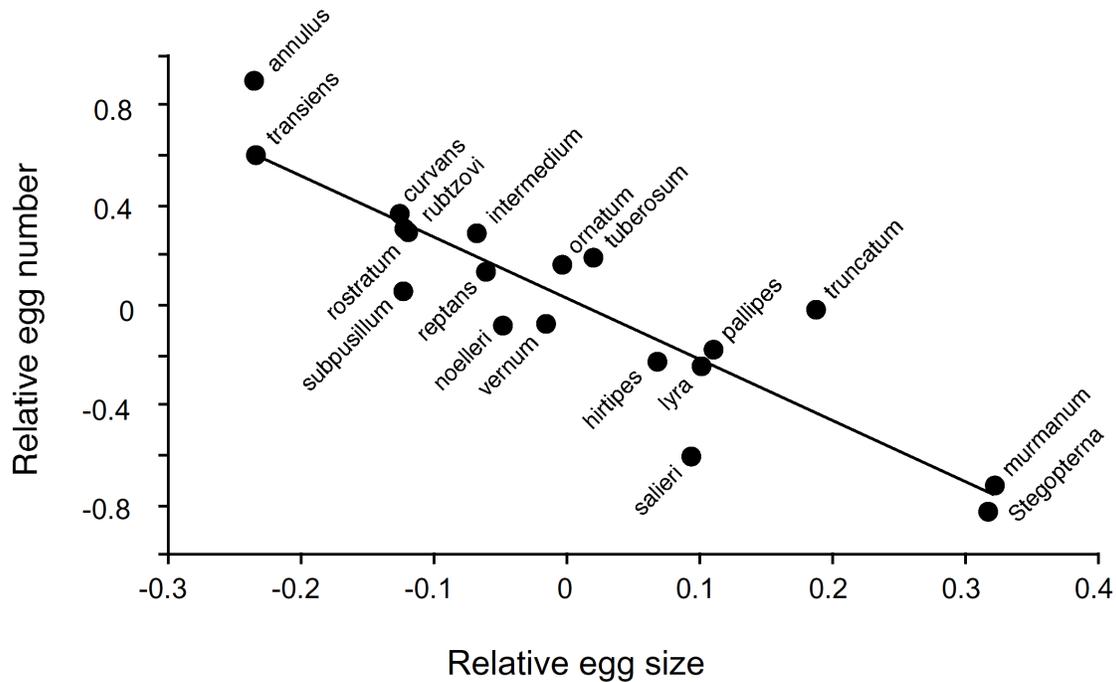
Figure 3. Tradeoff between egg number and size. The effects of body size are removed.

with repeated cycles of reproduction that depend on adult feeding to complete egg maturation, or parental care, such patterns generally are not found. Failure to demonstrate tradeoffs does not mean that they are lacking but rather that they are more difficult to show when assuming that the quantity of resources allocated to reproduction is constant, which not always is the case (Glazier 1992, Fox and Czesak 2000). Our finding suggests, however, that in black flies this quantity is largely constant and consistent with the original model by Smith and Fretwell (1974), indicating that environmental constraints do not vary much across species and that allometric relationships tend to be constant (Hard and Bradshaw 1993).

Why then are some species of black flies producing many small eggs and others few large eggs? Small eggs are assumed (rarely tested) to hatch into small offspring that suffer higher mortality than larger offspring (Elnum and Fleming 2000). Such a strategy might be associated with relatively short egg but extended larval developmental time, as in the case of overwintering species (Figure 2), but it also could reflect a habitat of relatively high stability or high risk of egg predation. Large eggs, on the other hand, are assumed to improve the ability to withstand environmental stress, including larval competition (Azevedo et al. 1997), desiccation

(Sota and Mogi 1992), and starvation (Solbreck et al. 1989, Mappes et al. 1997). Hence, one would expect that species in relatively disturbed habitats, or with low egg predation risk, would have large eggs. However, inspection of Table 1 shows that the species with large eggs are predominantly prosimuliines (*Prosimulium*) and basal simuliines (*Stegopterna*, *Cnephia*, *Metacnephia*), indicating a phylogenetic component. Also, it is not obvious why *S. murmanum* has large eggs or why other species (*S. aureum*, *S. rubtzovi*, and *S. reptans*) have small eggs. All the large-egg species develop early in the season, perhaps as a consequence of having large eggs. The adaptive advantage of early emergence is not clear but might be related to factors such as predation risk, host availability, and mating opportunity.

Comparing propagule metrics within species rather than across species may be more relevant in an evolutionary context, because such metrics are evaluated by selection pressure, directly related to fitness (Bernardo 1996). Regarding within-species tradeoffs, the virtual lack of significance suggests that the assumptions behind a tradeoff might not have been met (Bernardo 1996). For example, the resources assumed available to the mother might not have been locally limiting. As with the almost complete absence of tradeoffs between egg number and size within species, the relationships between

body size and egg number were weak, although positive for ten species. Relationships were stronger for some species than others, reflecting, with some exceptions, mean body size. Among these species, *C. pallipes* is large and the only autogenous species. Two of the smallest species, *S. reptans* and *S. transiens*, demonstrated the lowest rates of increasing egg size with body size. The small *Stegopterna* sp. and *Simulium subpusillum* failed to show a positive relationship. The reason for this pattern is unclear but is unrelated to variance in species size.

As expected, we did not find a difference between mammalophilic and ornithophilic species with respect to egg number and size. Blood feeding in these groups of species is likely to have similar constraints. Host type could, however, play a role. For example, the quality of blood is different, as stated in the introduction, and species that feed on large, sparsely distributed hosts, whether mammal or bird, should face higher costs, leading to a lower reproductive contribution than species requiring blood meals from more common hosts. However, nothing in our results supports any hypothesis other than the null hypothesis. In contradistinction, Gryaznov (1995) found that newly emerged females of ornithophilic species had more eggs (immature) than did those of mammalophilic species in western Russia. Thus, although the potential fecundity might be greater in ornithophilic species, the advantage might not always be reflected in actual fecundity (i.e., the number of mature eggs of a single egg batch).

One reason for weak relationships might be the heterogeneity in the collected material. For example, some females of the same species might have been captured carrying their first batch of eggs, while others were carrying subsequent batches. A decrease in egg-batch sizes with each cycle (Lewis 1958, Abdelnur 1968, Mokry 1980b) might have influenced the observed patterns. The inability to identify all females to species is somewhat problematic, although the inseparable species were always close relatives. Oviposition of partial egg batches before capture is another potential source of variation, but less likely, because eggs in a batch mature simultaneously and it seems improbable that the female would pause and return to terrestrial environments kilometres away only to return later for continued oviposition. However, it is possible that reduced volumes of blood have been ingested due to interrupted blood-feeding as a result of host defensive behavior. Crosskey (1990), however, argued that interruptions during blood feeding are unusual. In contrast, parasitism is a possible source of variation (Ham and Banya 1984, Branco 1999). Using only females with clearly expanded abdomens marginally improved the relationships between egg numbers and size, which suggests that the weak relationships are not primarily a consequence of partial egg batches. Further, some variation might occur with respect to spatial and temporal scales, as in the change of egg size with season. Most species in our study increased their egg size over time, in accordance with reported trends in invertebrates (Roff 1992).

The high natural variation observed in egg number and size in wild populations of black flies in northern Sweden is a qualitative result that requires further study. Given the high variation resulting from the above and probably other factors, as well as the use of field-caught material, the trends in general are surprisingly clear and the testing of the various hypotheses is, therefore, relatively robust. The current difficulty of maintaining adult black flies in the laboratory (Edman and Simmons 1987) provides few alternatives to collecting females in nature to study the problems of egg number and size.

Black flies show a remarkable capacity to adapt to a broad range of habitats. Although this capacity is a striking feature of larvae, for which much of the success can be traced to flexibility in the design of the food-capturing structures (Zhang and Malmqvist 1996, Malmqvist et al. 1999, Palmer and Craig 2000), such a capacity is less obvious in adult black flies. The reason is not that adults lack such adaptability but rather that it is more complex. During a limited time period, adult black flies emerge from streams and rivers, mate, feed on carbohydrate-rich food sources, and in the case of species requiring blood for one or more gonotrophic cycles, search for hosts, acquire blood, return to suitable water bodies, and locate optimal sites for oviposition (Sutcliffe 1986, Crosskey 1990). These processes are tackled by the females through a number of strategic choices. In this paper, we have examined how some of these choices are related to fecundity among species and, to a lesser degree, within species. In so doing, we believe that the success of adult black flies can be explained by the combination of some simple principles and a high flexibility.

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