U C L Université catholique de Louvain

Faculté des sciences
Unité d'écologie et de biogéographie
Dr. Melanie Gibbs

Physiological mechanisms underlying oviposition plasticity in response to increased flight at high temperatures

Host : Prof. Bengt Karlsson,
Department of Zoology
Stockholm University
S-106 91 Stockholm
Sweden

Purpose of visit

1) To explore the physiological mechanisms underlying oviposition plasticity in response to flight at high temperature by examining ovarian dynamics.
2) For the applicant to learn new physiology techniques and facilitate the transfer of knowledge between the host institution and the institute of origin.

Description of work carried out

Rationale: For many indigenous species, fast changing anthropogenic environments are altering selection pressures and evolutionary ecologists are particularly interested in the response of life history traits to these changes [1]. Due to functional constraints, trade-offs between life history traits can occur and the strength of these trade-offs may influence the response of traits to new selection pressures [1]. Anthropogenic landscapes are typically characterised by intensive land-use, a decline in habitat quality, altered environmental (thermal) conditions and an increased habitat fragmentation [2-4]. Consequently flying insect species are often required to travel over longer distances across wider areas in search of resources [5]. Flying over larger distances in fragmented landscapes during routine movements (i.e. the daily movement of individuals during foraging and oviposition [6]) requires more time, energy and resources. Physiological constraints caused by overlap in the resources used during flight and reproduction can therefore result in a trade-off, with any resources used during flight no longer available for use in reproduction. A decline in the resources available for reproduction can directly impact on reproductive strategies and alter egg size and fecundity [7]. In fragmented landscapes both the average and maximum daily temperatures are higher [8]. Temperature changes in fragmented landscapes may have important consequences for several aspects of insect life history including reproductive output [9]. Increased metabolic rates at higher temperatures can deplete resource reserves more quickly and it is known that increased temperature can directly impact on egg (and oöcyte) size and fecundity in female butterflies [9,10]. Given that females in fragmented landscapes may be simultaneously subjected to increased temperature and longer flights whilst searching for oviposition resources, considering these effects in isolation may substantially underestimate the impact of habitat fragmentation on reproductive output because increased flight at high temperatures is likely to be more

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energetically costly than increased flight at lower temperatures. Clearly therefore, to gain a more reliable estimate of the impact of changes in landscape structure on insect reproductive success it is necessary to explore the combined effects of *both* increased temperature and increased flight. Although documenting oviposition patterns in response to flight and temperature are interesting, they are difficult to explain without knowledge of the physiological and developmental mechanisms underlying oviposition plasticity. Thus, by exploring several of the complex physiological parameters involved in oögenesis, the purpose of this visit was to determine the causal mechanisms behind these observed patterns.

Methods:

1) Larvae were reared (4 per plant) under common garden conditions (22^{o}C day, 22: 2 LD cycle) on *Dactylis glomerata*.
2) At eclosion adult females (n = 59) were weighed and divided into 4 treatment groups:

Treatment 1: High temperature (27^{o}C) and no forced flight
Treatment 2: High temperature and forced flight (3 x 5 mins; post mating but prior to the onset of oviposition i.e. day 0, and again on days 3 and 7 of oviposition)
Treatment 3: Low temperature (19^{o}C) and no forced flight
Treatment 4: Low temperature and forced flight.

3) To measure daily reproductive output: Each female per treatment group were allowed to oviposit for 10 days. Each day, mean egg mass and fecundity were recorded.
4) To measure lifetime reproductive investment: Females were monitored until death. Longevity was recorded as the number of days from eclosion until death. The total number of eggs laid by a female during her life was recorded.
5) Ten eggs per treatment group, per day of oviposition, were used to determine the water, carbon and nitrogen content of eggs (after [11]). This allowed us to determine whether egg resource content changes during oviposition.
6) To examine ovarian dynamics: On days 2, 4, 6 and 8 of oviposition 10 females per treatment group were sacrificed and their ovaries dissected. One ovary (per female) was used to measure; number of ovarioles, number of oöcytes per ovariole, proportion of chorionated oöcytes (after [10]).
7) 323 eggs (collected from each treatment group) were individually weighed and kept in individual containers to monitor hatching success and embryonic development time at 19^{o}C (20 eggs) and 27^{o}C (20 eggs). These measures enabled us to examine whether temperature induced egg size variation has consequences for embryogenesis and hatching success.

Description of main results obtained

Preliminary results:

1) There was a significant effect of temperature on lifetime fecundity, but no effect of flight, and no flight by temperature interaction (Two-way ANOVA; Table 1). Females ovipositing at high temperatures had significantly higher fecundities than

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females ovipositing at low temperature (mean (± SE); low temperature = 85.02 (11.74), high temperature = 133.13 (11.54)).
2) There was no effect of temperature or flight treatment on mean lifetime egg mass (Table 1).
3) There was a significant effect of temperature on longevity, but no effect of flight, and no flight by temperature interaction (Table 1). Females ovipositing at high temperatures had a significantly shorter lifespan than females at low temperature [mean (± SE); low temperature = 25.49 (1.48), high temperature = 15.07 (1.45)].
4) There was a significant effect of rearing temperature on embryonic development time, but no effect of egg mass and no egg mass by rearing temperature interaction (Two-way ANOVA; Table 2). Eggs at low temperatures had significantly longer embryonic development times than eggs at high temperature [mean (± SE); low temperature = 9.22 (0.097), high temperature = 6.60 (0.229)]. Overall hatching success was very low with only 112/323 hatching and 80% of the eggs that hatched did so at low temperature.

Melanie Gibbs returned to the home institute (Université catholique de Louvain, Belgium) on 31st July, and the following data collection/analyses are currently being performed in Belgium:

Data currently being analysed:

5) Mean daily reproductive output; to determine whether investment patterns during early reproduction (i.e. days 1-10 of oviposition) differ across treatment groups and to examine potential egg size-number trade-offs.

Currently these data are still being collected:

6) Egg water, carbon and nitrogen content: the egg samples have been sent away to an external company in Uppsala for analysis. These data will be emailed to Melanie Gibbs at the Université catholique de Louvain and subsequently used in analyses.
7) Ovarian dynamics: Samples are in the freezer at the Université catholique de Louvain and are being dissected and stained with trypan blue to measure; number of ovarioles, number of oöcytes per ovariole, proportion of chorionated oöcytes in relation to flight treatment and temperature.

Summary of preliminary results:

Relative to females ovipositing at high temperature, females ovipositing at low temperature had lower lifetime fecundities even though their lifespan was significantly extended. The finding that females at low temperature are unable to maximise their potential fecundity even though they have an extended lifespan, is interesting, and analysis of the ovarian dynamic data will enable us to determine whether this is due to low egg maturation rates at low temperatures and/or retention of mature oöcytes resulting in a large number of unlaid eggs at death. Contrary to our expectation, there was no difference in mean lifetime egg mass between low and high temperature treatment groups. Large eggs are hypothesised to be laid at higher temperatures because their higher surface/volume ratio would enable them to cope better with desiccation and result in a higher hatching success. Our preliminary data analyses do not support this hypothesis. Calculation of mean lifetime egg mass does, however, mask a large amount of across-day variation in egg mass, and analysis of daily reproductive investment patterns may therefore prove to be valuable. We were expecting

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differences in fecundity, egg mass and longevity across flight treatment groups, but the preliminary analyses show no significant flight effects. This is a surprising result, but further analyses of the data examining more complex interactions between traits may yield some further interesting findings.

Future collaboration with host

To investigate the effects of temperature and flight on adult immune function in *Pararge aegeria*.

Projected publications

Gibbs M., Van Dyck H. & Karlsson B. Physiological mechanisms underlying oviposition plasticity in response to increased flight at high temperatures in the speckled wood butterfly, *Pararge aegeria*. To be submitted to the Journal of Insect Physiology.

Other comments

Other activities during this exchange visit include:

1) Seminar given by Melanie Gibbs "Flight reduces egg size in *Pararge aegeria*: Are there consequences for offspring development?" 1 June 2009, Department Zoology, Stockholm University.

2) Attended an international scientific symposium "Darwin's Evolution" 19-20 May 2009, Naturhistoriska riksmuseet, Stockholm.

3) Attended the weekly Department Zoology seminar series, Stockholm University.

4) New techniques learnt: dissection of ovaries, immunostaining of oöcytes.

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Table 1: The effects of temperature and flight during oviposition on female reproduction and longevity.

Trait	Treatment	df	MS	F-ratio	P-value
Lifetime fecundity	Temperature	1	3412.19	8.55	**0.005**
	Flight	1	1277.86	0.32	0.547
	Interaction	1	8100.54	2.03	0.160
	Error	55	3992.11		
Mean lifetime egg mass	Temperature	1	0.003	1.88	0.176
	Flight	1	0.004	2.47	0.122
	Interaction	1	0.004	2.30	0.14
	Error	55	0.002		
Longevity	Temperature	1	1601.24	25.41	**<<0.001**
	Flight	1	36.61	0.58	0.449
	Interaction	1	0.008	<0.001	0.991
	Error	55	63.03		

Table 2: The effects of egg mass and rearing temperature on embryonic development time

Factor	df	MS	F-ratio	P-value
Egg mass	1	0.37	0.44	0.510
Rearing temperature	1	4.72	5.58	**0.020**
Interaction	1	1.55	1.83	0.179
Error	107	0.85		

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