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Effect of non-lethal sampling on life-history traits of the protected moth *Graellsia isabelae* (Lepidoptera: Saturniidae)

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Abstract. 1. Non-lethal genetic surveys in insects usually extract DNA from a leg or a piece of wing. Although preferable to lethal sampling, little is known about the effect of leg/wing non-lethal sampling on fitness-related traits.

2. *Graellsia isabelae* (Graells, 1849) is a European moth protected by the Habitats Directive and the Bern Convention. Conservation genetics surveys on this species should therefore use non-lethal sampling.

3. The present study aimed to (1) quantify the effects of both leg and hind-wing tail sampling on survivorship and reproductive behaviour of adult males and females, and (2) assess the quality and quantity of DNA obtained from those tissues.

4. Both hind-wing tails and mid-legs proved to be good sources of high quality nuclear and mitochondrial DNA. DNA concentration was significantly higher when extracted from a large (130 mm^2) piece of the hind-wing tails than from about half of the mid-leg. Using mark–release–recapture experiments with adults, it was found that neither mid-leg nor hind-wing tail sampling significantly reduced male survivorship or total number of matings. However, although mid-leg sampling did not significantly affect female survivorship, it had a negative effect on female mating success.

5. Wing-tail clipping on males appeared to be the best non-lethal sampling procedure for *G. isabelae*. It is a fast procedure, similar to natural wing impairment, and did not significantly affect survival or mating behaviour.

Key words. DNA source, *Graellsia isabelae*, Lepidoptera, life-history traits, mark–release–recapture, moth, non-lethal sampling, Saturniidae.

Introduction

Non-lethal sampling for DNA analyses is gradually becoming a commonly used procedure in animal conservation genetics. In vertebrates, non-lethal procedures are not only available, but highly optimised (Horvath *et al.*, 2005) and refined (Wasko *et al.*, 2003), especially regarding DNA source (Schmaltz *et al.*, 2006). Unfortunately, non-lethal sampling is not always a feasible option for small animals, such as some insect orders (but see Gregory & Rinderer, 2004; Watts *et al.*, 2005).

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Non-lethal genetic surveys in insects usually extract DNA from a leg (Lai & Pullin, 2004; Hadrys *et al.*, 2005; Jensen *et al.*, 2005; Watts *et al.*, 2006), although a piece of wing (Keyghobadi *et al.*, 1999; Lushai *et al.*, 2000; Châline *et al.*, 2004) and caterpillar frass and exuviae (Feinstein, 2004; Watts *et al.*, 2005) were also successfully used as a source of DNA. Although, in theory, preferable to lethal sampling, little is known about the effect of leg/wing non-lethal sampling on fitness-related traits. Fincke and Hadrys (2001) suggested that removing a part of a leg (tibia) did not significantly alter either female survivorship nor egg laying, or male mating ability and territory tenure in *Megaloprepus coerulatus*. Adults of this damselfly species occasionally lose their tibia naturally. Starks and Peters (2002) found that apparent survival of tibia-cut wasps (*Polistes* sp.) was lower than the controls, but it was not confirmed that treatment individuals perished. In fact, severed adults found in

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the nests performed usual tasks necessary for colony survival. Holehouse *et al.* (2003) quantitatively showed that tarsus sampling did not increase worker mortality or affect foraging efficiency of worker bumblebees (*Bombus terrestris*). Similarly, Châline *et al.* (2004) found no adverse effect of wing clipping on honey bee flying behaviour and mating success. More studies evaluating the impact of non-lethal DNA sampling techniques are needed as different taxa, and even sexes, may show diverse responses to these procedures.

Lepidoptera (moths and butterflies) are very sensitive to human perturbation and climate change (Parmesan, 1996). Indeed, this order of insects suffered a rapid decline in Europe during the 20th century (Van Swaay & Warren, 1999; Wenzel *et al.*, 2006). Most attention to conservation of moths has been paid to species or assemblages of macrolepidoptera (New, 2004). Several studies have used molecular markers to identify lineages deserving conservation priority, mainly in butterflies (Cassel & Tammaru, 2003; Lai & Pullin, 2004; Gompert *et al.*, 2006; Vila *et al.*, 2006) but also in moths (Legge *et al.*, 1996; Clarke & O'Dwyer, 2000; Clarke & Whyte, 2003; Wynne *et al.*, 2003). However, few previous studies have quantitatively assessed non-lethal sampling methods of DNA on Lepidoptera from a conservation genetics perspective (Lushai *et al.*, 2000).

The present study assessed the effectiveness and consequences of two non-lethal sampling protocols in adults of the Spanish moon moth, *Graellsia isabelae* (Graells, 1849) (Lepidoptera: Saturniidae). This large moth is protected by the Habitats Directive and the Bern Convention [revised by Procter and Harding (2005)], and included in the French and Spanish official red lists of endangered fauna (Ministerio de Medio Ambiente, 2006; Ministère de l'écologie et du développement durable, 2008). Despite this protective legislation, the latest IUCN Red List considered *G. isabelae* as 'data deficient' (IUCN, 2007). The natural history and ecology of *G. isabelae* is reasonably well known (Ylla Ullastre, 1997; Chefaoui & Lobo, 2007). However, genetic studies are needed to disentangle its intraspecific taxonomy and to estimate parameters related to conservation genetics. Therefore, prior survey testing which tissue would be most suitable to be used as a DNA source, as well as what consequences such tissue sampling would have on fitness-related traits was necessary.

The aim of this study was to identify which of the two tissues widely used in non-lethal sampling in Lepidoptera minimised the harm for individuals of *G. isabelae*. For this, the quality and quantity of DNA obtained from severed mid-legs and clipped hind-wing tails was assessed. Furthermore, we quantified the effects of both leg and hind-wing tail sampling on survivorship and reproductive behaviour of adults of this emblematic species.

Materials and methods

Quantifying DNA quantity and quality from different tissues

We compared the amount and quality of DNA obtained from one mid-leg and one hind-wing tail taken from 40 males, to investigate which tissue was the best source of DNA. Males were hand-netted in May 2007 in the French Alps, sampled, and released in the same spot where they were collected. The left mid-leg tibia and tarsus (approximately 15mm in length) were removed by using scissors. The same procedure was used to clip a fragment (~ 130 mm^2) of the right hind-wing tail. Each of the 40 severed mid-legs was kept in a tube containing 100% ethanol, whereas hind-wing tails were individually placed in paper envelopes and kept dry. Both samples, ethanol-preserved legs and dry hind-wing tails, were kept at –20 °C for 1 month until analysis. Each tissue sample was manually disrupted by using disposable plastic pestles and microtubes (VWR™ International Eurolab S.L., Barcelona, Spain). Physical homogenisation took 30 s per sample and was performed by the same researcher. Total genomic DNA was extracted from both mid-legs and hind-wing tails separately using a commercial kit (DNeasy® Tissue Kit, Qiagen, Ileia S.L., Madrid, Spain). A final volume of 100 μL of DNA per sample was obtained, because the last two elutions (buffer AE) were performed with 50 μL of buffer AE each.

To determine the quality of the genomic DNA extracted, 100 and 200 ng of DNA were loaded into lanes of a 0.8% TAE agarose gel subjected to electrophoresis at 50 V for 4 h. The gel was visualised under UV light after ethidium bromide staining. The molecular weight of the gDNA bands was determined by comparison to a 1 Kb DNA Ladder (Bioron GmbH, Ludwig-Shafen, Germany). In addition, a ND-1000 spectrophotometer was used (NanoDrop Technologies) to measure both the concentration and purity (absorbance value ratio 260 nm/280 nm) of DNA obtained from both mid-leg and hind-wing tail. We measured DNA concentrations and purity less than 24 h after extraction in order to avoid the effect of sample evaporation.

Furthermore, we quantified double-stranded polymerase chain reaction (PCR) success rate of both leg (12 individuals) and hind-wing tail DNA extractions (47 individuals) using 10 polymorphic microsatellites (M. Vila, N. Mari-Mena, S. H. Yen and C. Lopez-Vaamonde, unpublished data) and two mitochondrial markers: cytochrome oxidase *c* subunit 1 (COI), and control region (CR). For DNA extractions of legs, a 786 bp of the COI was amplified. For DNA extractions of wings, a 278-bp fragment of the CR was amplified. Reagents and protocols for mitochondrial PCR amplifications were described by Vila and Björklund (2004).

Any non-lethal sampling method should cause as little effect on fitness-related traits as possible. Accordingly, we investigated whether leg and wing sampling had any effect on survivorship and mating in *G. isabelae* adults. For this, three experiments were performed.

Experiment 1: effect of leg sampling on survival of males

Graellsia isabelae rearing. Several hundred larvae of *G. isabelae galliaegloria* were reared at INRA-Orléans in 2006, using a standard protocol (Collectif OPIE, 1998). Larval stock comes from the upper Durance (Hautes Alpes, France). Three hundred pupae were kept outdoors in a garden shed over winter. In all, 17 females and 102 adult males were forced to emerge on 1 May 2007 by placing overwintered pupae in a controlled temperature (CT) room at 25 °C and 100% relative humidity (RH).

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Mark–release–recapture. Leg-clipped male survivorship was monitored by recapturing treatment and control males between 1 and 9 May 2007. This time period was selected based on the average longevity for *G. isabelae* males (5.87 ± 3.06 days; Ylla Ullastre, 1997).

We randomly selected 51 laboratory-reared adult males that had recently emerged (2–3 h after emergence) to be sampled for DNA by leg sampling (treatment). Mid-leg tissue was removed as described above. Likewise, we randomly selected 51 non-sampled (control) adult males that were handled in the same way as sampled adult males, except that no tissue was taken. All adult males were individually marked using numbered tags (Opalithplättchen®) glued to the costal vein of the right forewing using Loctite® superglue (Goussard & Roques, 2007).

Both marked treatment and control males were immediately released in a 3 ha pine nursery at INRA-Orléans. All marking, DNA sampling, and release was carried out over 4 days (45 males released on 1 May, 34 males on 2 May, 18 males on 3 May, and 5 males on 4 May 2007). Adult males were released between 10.00 and 14.00 hours. This release site was chosen because it contained a plantation of 20-year–old *Pinus sylvestris* trees, the preferred host plant of *G. isabelae*. In addition, there were no major lights within the forest which could attract marked males and disrupt the experiment. Furthermore, the site was outside the natural distribution range of the species, so experimental females would be the only source of sexual pheromone.

Seventeen freshly emerged virgin females were used to recapture experimental males attracted by female pheromones. Females were kept in mesh cages [16 cm (H) × 32 cm (W) × 16 cm (D)] to allow aerial dispersion of sexual pheromone. The cages were hung on pine branches at 1.5–2 m height. Females attracted males from 21:30 to 22:45, as males mostly fly at dusk (Ylla Ullastre, 1997). Females were kept in cages to prevent mating. This was because once females mate, they stop attracting males, so a much larger (an unavailable) number of females would have been needed for the experiment.

Males attracted by virgin females were hand-netted. Their numbered tags were recorded and captured males were kept in a cage to be released at 23.00 hours. Males were always released at the same geographic site. However, the point where receptive females were placed varied slightly (∼100–200 m) every evening, depending on wind direction. This is because pheromone molecules are wind dispersed, so males attracted by calling females tend to fly against the wind.

Weather conditions were mostly favourable over the 9-day recapturing period (temperature range at dusk was 10–18 °C). However, heavy rain prevented female calling on 8 May.

Experiment 2: effect of leg sampling on survival and reproduction of females

We surveyed whether leg sampling posed any handicap to *G. isabelae* adult females concerning survivorship and/or mating. The experiment was performed between 10 and 18 May 2007 in the French Alps.

Graellsia isabelae rearing. Female pupae of *G. isabelae galliaegloria* obtained at INRA-Orléans (laboratory colony) were taken to our alpine field station in Argentière-la-Bessée (Hautes-Alpes, France) and placed at 25 °C and 100% RH for emergence.

Tissue sampling. After adult emergence, 28 females were randomly selected for leg sampling (treatment). Only 28 experimental females were used, since the remaining females were needed to continue the mass rearing of the laboratory colony at INRA-Orléans. As in experiment 1, the left mid-leg tibia and tarsus were removed. We randomly selected 28 non-sampled (control) adult females, handled in the same way as treatment females, except that no tissue was taken from them. All adult females were individually marked using numbered tags as described above.

Monitoring reproductive behaviour. Both treatment and control adult females were set in the wild at Queyras (Hautes Alps, France), where the species naturally occurs. We placed two pairs of treatment and control females in each experimental site. Each female was tied around the base of its two forewings with a piece of 50–70 cm of cotton string. The string was itself attached to a freshly cut branch of *P. sylvestris* to prevent females from escaping. The pine branch with the attached experimental female was fixed to a plywood platform [30 cm (L) × 50 cm (W)], at a height of 80 cm from the ground.

Experimental females were placed in the field from 21.30 until 23.00 hours to attract wild males and mate. Mating lasted on average 3–4 h (Ylla Ullastre, 1997). Mated couples (experimental female + attracted wild male) were placed in a cage and taken back to the field station laboratory. The next day, males were released back in the very site where they were captured. Unmated females were taken back to the field during subsequent nights until mating was accomplished or the experiment finished. Mated females had the strings removed and were placed in individual cages [12 cm (H) × 22 cm (W) × 13.5 cm (D)] containing a pine twig for egg laying. Female survivorship was quantified by recording the date when experimental females died. Mating success was estimated from the proportion of mated females. The experiment finished when all experimental females died. Eggs were kept at INRA-Orléans in order to continue the laboratory colony.

Experiment 3: effect of hind-wing tail sampling on male survival and reproduction

We surveyed whether wing sampling negatively affected survivorship and/or mating of *G. isabelae* adult males by monitoring the longevity and number of matings. Again, male and female individuals were reared at INRA-Orléans until emergence in 2008.

We took a piece (130 mm^2) of the right hind-wing tail from 12 laboratory-reared adult males (treatment) 2–3 h after their emergence. Likewise, 12 adult males (control) were handled in the same way as treatment males, except that no tissue was removed. All adult males were individually marked as described in experiment 1. Similarly, both treatment and control males were released in the pine nursery (INRA-Orléans). This procedure was carried out over 3 days in May 2008 (6 males released on

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13 May, 8 males on 14 May, and 10 males on 15 May). Small sample size in this case was due to a limited number of adults available, due to high larval mortality in 2007.

In order to proceed with recapture, 20 freshly emerged virgin females were used to attract experimental males with their pheromone. Each female was tethered around the base of its two forewings as previously described. The string was itself attached to a branch of *P. sylvestris* to prevent females from escaping. Females were placed in the field from 21.30 until 23.00 hours. Tethered females were allowed to mate so male mating success could be quantified. Mated couples were placed in a cage and taken back to the laboratory. Males were released in the same site where they were captured during the next day. Mated females were placed in individual cages with a pine twig for egg laying. Unmated females were taken back to the field in subsequent nights until mating was accomplished. Male survivorship was monitored by recapturing and identifying marked males between 13 and 18 May 2008, as well as recording whether they mated or not. There were showers and thunderstorms every evening.

Females in experiments 2 and 3 were tethered, whereas they were kept in mesh cages for experiment 1. This was due to the different objectives of the three experiments. Females kept in mesh cages were used as a tool to call for males and to estimate male survival. The advantage of using tethered females is that one can actually observe mating, which was one of the issues addressed in experiments 2 and 3.

Statistical analysis

For all experiments, all values are given as means ± SD unless otherwise stated. All tests were two-tailed and the level of statistical significance was $P = 0.01$. Non-parametric tests were used because, with the exception of the female survivorship dataset, the majority of datasets significantly deviated from normality (Kolmogorov–Smirnov test). SPSS Version 10 was used to conduct the statistical analyses.

Results

Tissue-dependent DNA yield

All DNA extractions were positive (>8 ng μl^{-1} for legs; >20 ng μl^{-1} for wings). DNA concentration was significantly larger for hind-wing tails [median (interquartile range) = 44.35 (18.7) ng μl^{-1}] than for mid-legs [17.4 (7.6) ng μl^{-1}], Wilcoxon signed ranks test: $Z = -5.5$, $n = 40$, $P < 0.001$. This was not surprising since the amount of tissue (dry weight) of hind-wing tails was higher than that of mid-legs (Supporting Information).

Both tissues yielded good quality DNA. On the one hand, gel electrophoresis showed sharp bands of high molecular weight with no evidence of degradation for both leg and wing DNA extractions (pictures available upon request). On the other hand, DNA purity, as estimated by using the 260 nm/280 nm ratio, ranged between 2.48 and 1.67 (median = 2.0) for mid-legs and 2.04 and 1.58 (median = 1.8) for hind-wing tails.

Overall, PCR success rate was 100% for both nuclear and mitochondrial genes. All 10 microsatellite loci amplified from wing tissue DNA extractions ($n = 47$) and only one out of 12 DNA extractions (91.67%) from leg tissue did not amplify. For mitochondrial DNA, we obtained 95.45% ($n = 132$) of successful amplifications from wing tissue (target gene: mtDNA CR), and 98.02% ($n = 101$) from leg tissue (target gene: mtDNA COI). All failed reactions worked correctly when PCR conditions (i.e. annealing temperature, magnesium concentration) were optimised.

Experiment 1: effect of leg sampling on males

Male recapture and survivorship. There was no significant difference between total number of treatment recaptured ($n = 42$) and control males ($n = 46$) (47.73% vs 52.27%; $\chi_1^2 = 0$, $P = 1$). Mean (±SD) number of males recaptured per day (7 ± 5.5, treatment; 6.6 ± 5.6, control) did not significantly differ either (*t*-test: $t_{11} = 0.139$, $P = 0.892$).

The percentage of males recaptured decreased with time in both treatments and controls. Thus, the first day after release the mean percentage of recaptured males for controls was 41.54%, whereas the third day the level was 7.82% (Fig. 1). Number of males recaptured dropped to 1.14% in the fourth night after release. Treatment males showed a similar decrease in the mean percentage of recaptured males (Fig. 1). The oldest male caught was 5 days old (male no. 11, control). This male was recaptured every night over five consecutive nights.

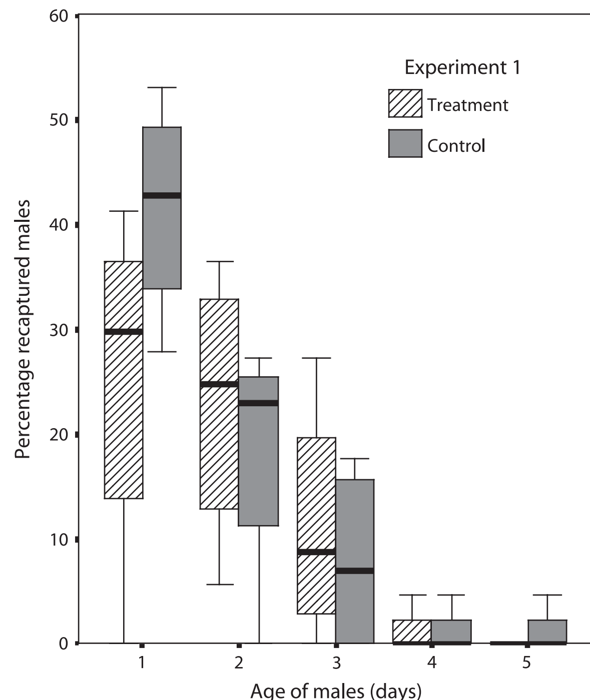

Fig. 1. Relationship between male age and the average percentage (±SE) of recaptures (experiment 1, $n = 191$ recaptures).

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There was no significant difference in male survivorship between treatment: [median (interquartile range) survivorship per male = 2 (1) days, $n = 28$ males] and control [2 (2) days, $n = 25$ males] (Mann–Whitney U-test: $U = 302.5$, $P = 0.37$).

Experiment 2: effect of leg sampling on females

Female survivorship. Two experimental females [no. 4 (treatment) and no. 3 (control)] disappeared in the field. Average survivorship of the remaining experimental females was 7.8 (2.3) days for treatment ($n = 26$ females) and 7.9 (1.7) days for control ($n = 26$ females). There was no significant difference in survivorship per female (paired t-test: $t_{25} = -0.15$, $P = 0.9$) (Fig. 2).

Female reproductive behaviour. Leg sampling had a negative effect on female mating success. The majority (59.3%) of treatment females (16 out of 27 females) did not mate compared to only 25.9% of control females (seven out of 27 females) ($\chi_1^2 = 0$, $P = 1$).

Experiment 3: effect of hind-wing tail sampling on male survival and reproduction

Male recapture and survivorship. There was no significant difference between total number of treatment males recaptured ($n = 9$) and control males ($n = 11$) (75% vs 91.7%; $\chi_1^2 = 0$, $P = 1$). The percentage of treatment males recaptured the first and second nights after release was 5% and 13%, respectively. For control males, the recapture rate was 10% for the first two nights. Neither treatment nor control males were recaptured after the third night. There was no significant difference in male survivorship between treatment [median (interquartile range) survivorship per male = 2 (1) days, $n = 7$ males] and control [2 (2) days, $n = 5$ males] (Mann–Whitney U-test: $U = 14.5$, $P = 0.60$).

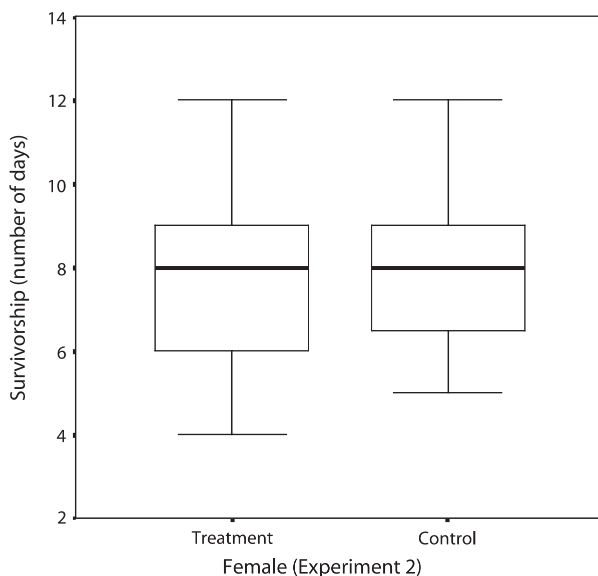

Fig. 2. Survivorship for 54 females of *Graellsia isabelae* (experiment 2).

Male mating success. We obtained a total of 11 mating pairs. Eleven females mated with eight different males. Five females mated with five treatment males and six females mated with three control males. Therefore, male mating seemed unaffected by hind-wing sampling with regard to total number of matings (five for clipped males and six for control males). It is worth noting that all females were singly mated, whereas some control males mated with several females. Indeed, one control male (no. 53) mated with three different females. All mated treatment males had a mating frequency of one. However, although treatment males showed a lower mating frequency than control males, this difference was not significant (Mann–Whitney U-test: $U = 2.5$, $P = 0.05$).

Discussion

Both hind-wing tails and mid-legs proved to be good sources of high quality nuclear and mitochondrial DNA. Keyghobadi *et al.* (1999) also obtained good quality and quantity of genomic DNA using a commercial kit from a wing piece of 15 mm^2. Lushai *et al.* (2000) obtained approximately 0.8–1 mg DNA when pooling two independent (TE buffer and phenol) extractions from a wing piece of about 3 mm^2. In the present study, hind-wing tails yielded twice as much DNA as mid-legs and of a similar purity. However, this was not surprising since (1) the amount of tissue taken from one hind-wing tail was higher than that of the fragment of a mid-leg, and (2) DNA content and amount of tissue used for extraction were correlated (Supporting Information). One might argue that a good way of increasing the yield of DNA extractions from leg tissue might be to also use the femur. We did not sever this part, because the sampling procedure would be risky for the individual, as the femur in *Graellsia* is relatively short and the thorax very hairy.

Wing tail clipping appeared to be the best tissue sampling method tested in the present study. Besides yielding high and good quality DNA content, wing tail clipping had no significant negative effects on either survivorship or mating success of adult males. Indeed, male mating seemed unaffected by wing tail sampling with regard to total number of matings (five for clipped and six for control). Another advantage of using wing tails is that their loss is unlikely to cause any artificial negative effect on fitness, as they are often worn out after a few days of flight. Indeed, Roland *et al.* (2000) showed that wing clipping affected neither maximum flight distance nor survival in the papilionid butterfly *Parnassius smintheus* regardless of sex. This lack of effect between wing manipulation and survival agrees with prior findings of no relation between natural wing polymorphism and survival (Solbreck, 1986; Denno *et al.*, 2001).

Interestingly, our experiment confirmed multiple mating for males (Ylla Ullastre, 1997), as three control males mated with six females. Hind-wing clipped males showed similar survivorship and a slightly lower mating frequency per male than control ones. The differences in male survivorship and mating success were not significant. However, sample sizes are rather small and therefore, more data is needed before drawing further conclusions.

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Also, it will be interesting to determine the effect of wing clipping on survivorship and mating success of females, as many calculations in the field of conservation genetics would benefit from having data on both sexes (e.g. dispersal ability).

In contrast, leg sampling had a negative effect on mating success of females, probably due to the stress caused to females during the sampling process. However, our findings may not accurately reflect actual female survivorship or reproductive behaviour, as tethered females may have also been stressed. We decided to tether females to prevent them from escaping. Despite this precaution, two females disappeared during experiment 2. They may have been predated or blown away by strong winds.

Both experiments 1 and 3 showed a fairly high proportion of male recapture in the first and second nights after release when compared to similar studies in butterflies (e.g. Schmitt *et al.*, 2006; Samways & Lu, 2007) and moths (e.g. Cameron *et al.*, 2002; Mo *et al.*, 2003). However, our study is based on a much lower number of marked individuals (experiment 1: 102 marked males; experiment 3: 24 marked males) than the studies mentioned above (e.g. Schmitt *et al.* based their survey on 2211 marked individuals). Data from experiment 1 showed the high attractiveness of the sexual pheromone in *G. isabelae* and suggested that males of this species are likely to disperse over long distances. This is illustrated by the fact that one male was recaptured over five consecutive nights. It could be argued that our level of male recapture in experiment 1 was unusually high due to the fact that the experiment was carried out in Orléans (where *Graellsia* does not naturally occur), so experimental males were only attracted by the experimental females. However, similar mark–release–recapture experiments performed in the French Alps gave a similar percentage (38.71) of male recapture (Goussard & Roques, 2007). This high level of male recapture will facilitate future demographic studies that aim order to estimate male dispersal ability in this species.

The present study provides an appropriate non-lethal tissue sampling protocol for adults of *G. isabelae*. Wing-tail clipping appeared to be the best DNA source in males: it yielded high and good quality DNA content without significant negative effects on fitness. It will also be interesting to determine if wing clipping has any influence on female reproductive behaviour, as leg sampling does. These surveys will be of great value for population genetics surveys aiming at revising the conservation status of this highly protected moth. Caution should be taken when extending our results to other species, as the presented sampling protocols (1) need further testing concerning their effect on females and other fitness-related traits, and (2) are not likely to be generally applicable to other species, in particular butterflies or moths without or with much reduced wing tails (e.g. 130 mm^2 of wing tissue may be too invasive for some insects).

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Supporting Information

The following supporting information are available for this article:

Besides the amount of tissue, one might argue that the difference in DNA content found in our study was due to a putative negative effect of ethanol on the preservation of DNA. To investigate this, as well as to estimate the correlation between amount of tissue and DNA content, we performed a new set of DNA extractions (GenElute™ Mammalian Genomic DNA Miniprep, Sigma-Aldrich Co., St. Louis, mo) from known amounts (dry weight) of tissue. DNA content and amount of tissue were positively correlated (Pearson's $r = 0.57$). Ethanol did not appear as a negative factor regarding DNA preservation of wing tissue. Results for descriptive variables related to quantity and quality of DNA given in the additional supporting information.

Additional Supporting Information may be found in the online version of this article.

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