Local adaptation and evolutionary potential of multi-host parasites

by

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Master of Science

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Supervisor

Abstract

In this thesis I test predictions relating to two problems in parasite ecology. First, I test whether host species range influences the likelihood of finding local parasite adaptation and second, whether the abundance of a given host species locally determines parasite traits or fitness. Using a novel statistical analysis and a critical literature review, I found that parasites with many host species were less likely to be adapted to local populations of single host species, after correcting for differences in experimental approaches and other factors that may have confounded results. Using empirical approaches and detailed field studies, I found that the water mite *Limnochares americana* Lundblad, a parasite with many dragonfly hosts, appears to track an abundant host, *Leucorrhinia frigida* Hagen, and has higher fitness on this host compared to a less abundant host *Nannothemis bella* (Uhler). Together, these findings indicate that use of multiple host species has implications for testing local adaptation theory.

Acknowledgements

From what I understand about scientific writing is that it is necessary for textual arrangements to suggest a notion of surplus. I have been able to comply with this convention in my thesis. I would not, however, been able to define such a sense of potential and possibility if it was not for the supervision of Dr. Mark Forbes. Mark has shaped all aspects of my understanding in biology, but more importantly, has directed my desire to approach and pursue ideas (he alone can be accredited for this). It is not possible to capture my respect for him through printed word; this is the failure of language.

The point of this text is to understand the connection between the history behind this thesis and the body of this thesis; both however I believe are flawed. "Knowledge is not made for understanding, it [should be made] for cutting", although Michel Foucault was likely referring to a different 'knowledge', I still think his quote (or my misquote) best summarises the contents of this thesis (and I'll leave it at that). I recall experiencing two major cohorts of 'Forbians' (forgive me if I've forgotten some). The first included Dave McRuer (knight of the lab-javelin, or was it captain jack-ass; I can't remember), Dean McCurdy, Heather McCracken and Christopher Yourth (to which I accredit my field 'experience' with dragonflies). The second included Tonia Robb and Erin Arnold (both masters of nuisance and general contributors to my suffering and morose). Thanks to all for letting me be a "poor bastard."

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CHAPTER 1

GENERAL INTRODUCTION

Theory predicts that the disproportionate evolutionary success of parasites should lead to selection for specialisation on host populations (Ebert 1994; Thompson 1994; details in Chapter 2). Parasites are thought to succeed more than their hosts because parasites generally have shorter generation times than their hosts, as well as greater reproductive output, larger populations, greater migration rates, or even higher mutation rates (see Hafner *et al.* 1996; Hamilton *et al.* 1990; LeClerc *et al.* 1996). These factors are thought to help accelerate the turnover of novel and successful parasite variants on host populations (Kaltz *et al.* 1999). This coevolutionary process¹ is predicted to result in parasites becoming significantly more infective or successful on local hosts than on non-local hosts of the same species (a hypothesis known as local parasite adaptation; Ebert & Hamilton 1996; Lively 1999).

¹ Coevolution, *sensu stricto*, reciprocal genetic change, over time, in pairwise interacting species (see Thompson 1994). Not considered explicitly in this thesis, are variations on coevolution or alternative hypotheses, such as stepwise coevolution (see Berenbaum 1982), sequential evolution (see Jermy 1984), or parallel cladistics (see Farrell 1998).

Experiments testing for local adaptation, however, often produce equivocal results (Gandon *et al.* 1996; Roy 1998; see also Table 1.1 for recent research outcomes). Many studies have supported local adaptation (e.g., Ebert 1994; Lively & Dybdahl 2000); whereas, others have provided counter-evidence for the hypothesis (i.e. parasites show greater success on non-local hosts; e.g., Kaltz *et al.* 1999; Oppliger *et al.* 1999). Other studies have shown that parasites have equal success on local and non-local hosts (e.g., Davelos *et al.* 1996; Multikainen *et al.* 2000). These results challenge the conventional wisdom that parasites have greater evolutionary potential than hosts, and also suggest that parasites may not often precede hosts in coevolutionary arms-races.

Recent approaches have applied spatial structure to coevolutionary interactions in effort to understand better the emerging patterns of parasite adaptation (Frank 1997; Gomulkiewicz *et al.* 2000; Nuismer *et al.* 1999). Here, local adaptation is scale dependent and may only occur at scales larger than parasite or host populations inter-connected by gene flow (Gandon *et al.* 1996; Morand *et al.* 1996; Lively 1999; Gandon 2002). Patterns of local adaptation may be affected more by differential migration rates of parasites and hosts, rather than by conventional factors thought to affect evolutionary potential; but see Gandon & Michalakis (2002) for an attempt to unite these factors. There are several logistical challenges, however, in measuring gene flow of parasites and their hosts, and this has impaired empirical tests of predictions from any meta-population model of local adaptation.

Another factor that is often neglected, but that should also influence coevolutionary potential of parasites, is host species use. Many parasite species are known to exploit multiple species of hosts. For instance, 94% of avian brood parasites have more than one host species (M. J. Lajeunesse, *unpublished data*), and 80% of pathogens of domestic animals exploit several hosts (Cleaveland *et al.* 2001). In fact, only a minority of parasites appear to be strict specialists (i.e. exploiting only a single host species; Woolhouse *et al.* 2001). One consequence of exploiting multiple host species can be a reduction in the turnover of successful variants on local host populations. Thus, the number of host species exploited by a parasite, at a particular locale, may affect ability and rate of parasites to track primary hosts (i.e. those that are true targets of selection; Futuyma & Slatkin 1983; Thompson 1994). Variation in spatial and temporal abundance of hosts should further dictate which host species will become targets of parasite selection.

The central objective of my thesis is to explore the conceptual linkages between patterns of host use and rates of adaptation on local hosts. More specifically, I evaluate two hypotheses: (1) use of multiple host species may impede adaptation to local host populations; and (2) relative abundance and interaction with particular species of hosts will affect the degree of selection imposed by these local hosts on local parasites. To evaluate these hypotheses, I first review the literature on local adaptation and test whether host species range influences the likelihood of parasites locally adapting to local host populations, using analyses akin to meta-analysis in ecology (Chapter 2). I then test the premise that parasites with multiple host species should be selected to use

abundant host species and exploit them more effectively than secondary hosts (those not primary targets of selection), using an empirical study. In this second test, I used a parasitic water mite, *Limnochares americana*, and examine patterns of host use on two of its dragonfly hosts, *Leucorrhinia frigida* and *Nannothemis bella* (with the former being the most common host; Chapter 3). My ultimate goal is to show how these problems in parasite ecology are linked and how tests of local parasite adaptation should proceed in light of the fact that parasites with multiple hosts are often considered for study, but their interactions with primary and secondary hosts are not considered explicitly.

TABLE 1.1. The methods and rationales for experimental approaches used to test for local adaptation in parasites. Despite fundamental differences between each approach, both should provide evidence for local adaptation, should it exist. Method (1) is most commonly used. Actual results refers to the outcomes from each study seen in the literature prior to my investigation (for details of studies see Table 2.1).

method	rationale	actual results		
		local adaptation	null outcome	local maladaptation
(1) compare the success of a parasite on different host populations	local adaptation occurs when the success of the parasite on local host populations is greater, on average, than its success on non-local host populations	parasites succeed better on local than non-local hosts	no significant differences in success of parasites on local and non-local hosts	parasites succeed better on non-local than local hosts
(2) compare the success of a parasite on local host populations with that of the parasite, from another population, tested on the same host population	local adaptation occurs when the success of the local parasite is greater, on average, than the success of the non-local parasite on local host populations	local parasites succeed better than non-local parasites on local hosts	no significant differences in success between local versus non-local parasites on local hosts	non-local parasites succeed better than local parasites on local hosts

CHAPTER 2

HOST RANGE AND LOCAL PARASITE ADAPTATION

2.1 Abstract

Parasites may be expected to become locally adapted to their hosts. However, while many empirical studies have demonstrated local parasite adaptation, others have failed to demonstrate it, or have shown local parasite maladaptation (see Chapter 1). Researchers have suggested that gene flow can swamp local parasite-host dynamics and produce local adaptation only at certain geographical scales; others have argued that evolutionary lags can account for both null and maladaptive results. In this chapter, I use item response theory (an analogue to meta-analysis) to test whether host species range influences the likelihood of parasites adapting locally to their host populations. I collated 32 independent experiments testing for local adaptation, where parasites could be assigned as having either broad or narrow host species ranges (BHR and NHR, respectively). Twenty-five tests based on BHR parasites had a significantly lower average effect size than seven NHR tests, indicating that studies based on BHR parasites are less likely to demonstrate local parasite adaptation. I argue that this may relate to evolutionary lags during diffuse coevolution of BHR parasites with their hosts, rather than differences in experimental approaches or other confounding factors between BHR and NHR studies.

2.2 Introduction

Parasites may be expected to become adapted to their local hosts because parasites are often more numerous and have shorter generation times than their hosts (Hamilton *et al.* 1990; Ebert 1994; Ebert & Hamilton 1996; Imhoof & Schmid-Hempel 1998). Thus, parasites should evolve faster than hosts in ways to increase their fitness at the expense of local hosts. Although parasite local adaptation is common, many studies fail to demonstrate it (Strauss 1997; Koskela *et al.* 2000), whereas others detect local parasite maladaptation (Kaltz *et al.* 1999; Oppliger *et al.* 1999). Some researchers have thus concluded that local parasite adaptation occurs only on average (see Kaltz & Shykoff 1998; Van Zandt & Mopper 1998). Recently, Lively (1999) suggested that short generation times of parasites are neither necessary nor sufficient for local adaptation. It is known that some parasites are not locally adapted despite having much shorter generation times than their hosts (Memmot *et al.* 1995; Kimberling & Price 1996; Alstad 1998).

Collectively, these studies suggest that other aspects of parasite natural history may be important determinants of local adaptation. Evolutionary lags between parasite genotypes tracking host genotypes may account for some parasites being able to exploit allopatric (non-local) hosts better than sympatric (local) hosts (Morand *et al.* 1996). Additionally, migration events may swamp local parasite–host dynamics (Ebert 1994; Gandon *et al.* 1996; Lively 1999; Nuismer *et al.* 1999), such that parasite adaptation is evident only at particular geographic scales (Hanks & Denno 1994; Imhoof & Schmid-Hempel 1998). However,

comparative migration data across diverse parasite-host associations are scant, making it difficult to test for the effects of gene flow.

Another possible predictor is host species range or the number of host species exploited by the parasite (hereafter referred to as host range). Documenting host range and/or the degree to which particular host species are exploited requires detailed field investigation or published accounts. For instance, the hen flea (*Ceratophyllus gallinae*) is known to parasitize at least 75 bird species (Tripet & Richner 1997; Table 2.1). My contention is that exposure to several host species may weaken species-specific selection, such that the parasite's ability to adapt to any particular host species is depressed (both at the population and at the species level). For parasites with broad host ranges (BHR), such as the hen flea, various host species might influence the coevolutionary trajectory (and lags) of the parasite. As such, host range would make the exhibition of local adaptation difficult or non-apparent at certain scales (Gomulkiewicz *et al.* 2000), or problematic when non-tracked hosts are chosen for experimentation. I hypothesise that host range variation contributes to the disparity of results for tests of local adaptation.

To explore this, I compare differences in fitness for local and non-local parasites from transplant experiments, for parasites differing in host range. I also consider different types of parasite–host associations (plants and plant pathogens, invertebrates and their parasites, etc.). Transplant experimentation is the most common method for comparing 'performance' of parasites (infectivity or within-host growth rate and survival) on local and non-local hosts, usually from different host

populations (Kaltz & Shykoff 1998; Van Zandt & Mopper 1998). This study provides the first general test of whether host range relates to local adaptation, using analyses based on item response theory (IRT). I show that parasites with a few to several well-documented hosts are less likely to support local adaptation than parasites with principally one well-documented host, most probably because parasites (in the former category) enter into diffuse coevolutionary interactions². Such findings have implications for studies on predicted impacts of parasitic or disease organisms on existing and novel hosts.

2.3 Methods

2.3 a) *Data collection and selection criteria*

I collated information from 32 independent tests from papers published on local adaptation and references cited in review papers (Boecklen & Mopper 1998; Gandon 1998; Kaltz & Shykoff 1998; Van Zandt & Mopper 1998). My search was exhaustive, but limited to published accounts (literature searches from 1979-2001). Many papers failed to show local adaptation. In fact, there were nearly as many null as positive results (Table 2.1), indicating that poor representation of null results did not occur. I would have run into the associated "file drawer problem" (Arnqvist & Wooster 1995) only if null studies based on parasites with narrow host ranges (NHR) were less likely to be published than null studies based on parasites with BHR; see Van Zandt & Mopper (1998) for a similar rationale. Although less

² Diffuse coevolution, interactions with secondary or ancillary host species are needed to understand the coevolutionary dynamics of primary interactions.

represented in the literature, negative results (local maladaptation) were also available for analysis.

I used IRT to estimate effect sizes (detailed below) rather than meta-analysis. Meta-analysis requires that a common currency is either measured or can be readily computed (see Goldberg *et al.* 1999). Metrics of parasite fitness were extremely variable among studies (e.g., parasite infection rates, growth rate, survival, spore production), and even included host-centred measures (e.g., condition, survival; cf. Mutikainen *et al.* 2000). While all measures are (thought to be) surrogates of fitness, they are not linearly equatable across studies as required by meta-analysis (Hedges & Olkin 1985). As such, measures of parasite performance were comparable only within studies. This general problem of using meta-analysis was further exacerbated by the variable reporting of means, standard deviations, and test statistics that reflected, in part, nuances of experiments, but that made computing traditional effect sizes and statistical conversions difficult (e.g., Arnqvist & Wooster 1995). I felt that many studies, where host ranges were obtainable, would have to be omitted if I used meta-analysis.

To be included in my analyses, tests had to compare metrics of parasite fitness (either parasite- or host-centred) for parasites exploiting local and non-local hosts of the same species, using a transplant or reciprocal transplant experiment (see Figure 2.1, but see also Table 1.1 for a second approach). In the latter case, this would produce two local–local controls and two local–non-local experimental groups (see Figure 2.1a). Studies that compared the performance of parasites on

different host species (one local and one non-local) were excluded if they did not complete the reciprocal comparison among populations of the same host species (e.g., Akimoto 1990; Via 1991). This exclusion was necessary because NHR parasites (see below) were not likely to be tested on different host species.

Metrics of parasite fitness also had to be compared among groups, preferably in a common garden setting (i.e. all experimental treatments were exposed to similar environments; see Lively 1989; Table 2.1), although this was not always possible for practical reasons (e.g., Briskie *et al.* 1992). Additionally, replication had to be indicated, such that sample sizes for comparisons of allopatric and sympatric test associations could be assigned. I also required that two-tailed tests (alpha 0.05) for parasite/host performance between sympatric and allopatric associations were reported, given that null outcomes and local maladaptation were also possible.

Only empirical studies were included (i.e. not theoretical treatments; e.g., Kraaijeveld & Godfray 2001). Finally, I only included studies if parasites could be assigned a host range (e.g., excluding studies like Oppliger *et al.* 1999). Host range was assigned by detailed literature searches; I did not rely on labels such as 'specialist' or 'generalist'.

Parasites with a NHR were those that have a single well-documented host species, or a single host species at the stage of their life cycle being studied (i.e. for macrocyclic trematodes, fungi, etc.). In some cases, there were reports of anecdotal associations between parasites and novel hosts (i.e. hosts not normally encountered in nature). I treated these as NHR parasites (although they were described as having two hosts; Table 2.1), while recognising that such unnatural

associations also would occur for parasites with a BHR. Parasites with BHRs had at least three (and most often many more) well documented hosts for the life stage being considered (Table 2.1). This classification of NHR and BHR parasites may seem somewhat artificial, because NHR parasites in my study may be shown to have more hosts through more detailed parasitological work. Likewise, BHR parasites may have rather narrow host ranges over much of their geographical range, and thus could be treated as NHR parasites should this degree of spatial variation in host use become available. Host range of BHR and NHR parasites does not give an indication of the age of association or the degree of interaction with each host species; as all hosts (either primary, secondary and/or ancillary) are treated equally. However, I do feel that this classification reflects the degree to which parasites considered herein enter 'diffused' coevolutionary relationships with hosts. Finally, I treated results for different host–parasite associations as independent, even if either the host or the parasite was shared with another association; see Poulin (1996) and Schalk & Forbes (1997) for similar rationale(s).

2.3 b) Analyses

To estimate effect size for NHR and BHR studies, I used IRT (Lord 1980; van der Linden & Hambleton 1997; Appendix A). In this study, a data point (k) resulted from a two-tailed test (or multiple tests) for differences in parasite performance on sympatric versus allopatric hosts. Data points were scored as either '0' if there was no significant difference in parasite performance on local versus non-local hosts, '1' if there was significant effect indicating parasite local

adaptation, or '-1' if parasites showed local maladaptation (see Table 2.1). I scored a study's research outcome by evaluating whether all tests showing significance in a particular direction (either towards local adaptation or maladaptation) were not attributable by chance, after adjusting for the total number of tests examined in that particular study using binomial expansion (see Appendix A). Here, the probability that such results could be due to chance decreased as the number of significant test results increased. No study showed evidence for local adaptation on one metric and evidence for local maladaptation on another. Studies showing no effects on all metrics were categorised as null studies. While this approach biases toward concluding local adaptation, I note that that it should not be more likely for researchers testing NHR versus BHR parasites. As mentioned, many researchers did not conclude local adaptation and I wanted to know whether those researchers more often dealt with BHR parasites.

I further contend that the use of p -values to help score outcomes of studies is valid based on IRT (despite not being valid for typical meta-analyses; Gurevitch & Hedges 1999; Osenberg *et al.* 1999). I note that results are weighted by corrections based on sample sizes (see below), so significant results based on small samples are given low weight, avoiding the funnel problem often seen in meta-analysis (i.e. precision of study result, and fixation on actual result, is relative to its sample size). Moreover, research outcomes are not directly based on p -values, but on the relative probability that all significant tests were not due to random effects. IRT was initially developed to adjust psychometric test scores for the effects of 'nuisance' properties (such as liability to guessing, or question

difficulty; van der Linden & Hambleton 1997). However, if the probability of answering test questions correctly (influenced by their 'difficulty') and the responses from a testee are known, then IRT permits the estimation of that testee's 'ability'. I use IRT to estimate the effect (analogous to 'ability') of BHR and NHR studies to show local adaptation (Appendix A).

I recorded a vector of research outcomes for NHR and BHR studies (scoring method above), and assigned a 'likelihood' of exhibiting local adaptation to each constituent study (weighted by sample sizes and adjusted for multiple tests; Appendix A). Here, effect sizes are scale-free estimates of the degree to which particular types of studies support local adaptation. Local maladaptation outcomes will lower average responses more than null outcomes. I feel this is appropriate, since the main prediction is that parasites should do better on local hosts; if they do worse, this should be reflected in scores. In summary, this procedure allows estimation of effect sizes and confidence limits for groups of studies, where studies have polytomous outcomes and unequal sample sizes (Appendix A).

My main test was whether BHR and NHR parasites differ in likelihood of showing local adaptation. Thus, I partitioned the dataset into NHR and BHR tests and computed effect sizes and confidence limits for these two types of studies. I then excluded certain parasite–host associations to ascertain whether any overall results might be ascribed to just one or a few types of associations. Some associations have figured prominently in tests of local adaptation (e.g., the adaptive deme formation of phytophagous insects; see Mopper & Strauss 1998).

2.4 Results

The overall effect size did not significantly differ from zero (Table 2.2), denoting a general likelihood of showing local adaptation half the time, as concluded by others (cf. Kaltz & Shykoff 1998; Van Zandt & Mopper 1998). Studies based on BHR and NHR parasites differed in the degree to which they supported local adaptation (Scheffé's contrast: $\chi^2 = 6.06$, d.f. = 1, $p = 0.014$). Specifically, studies examining BHR parasites had a negative effect significantly different from zero, while studies examining NHR parasites showed a weak positive effect (Table 2.2). These differences were not attributable to differences in sample sizes between BHR and NHR studies ($\chi^2 = 0.46$, d.f. = 1, $p = 0.49$), nor influenced by a U-shaped trend in sample sizes versus research outcome (i.e. 1, 0, -1) ($F = 0.65$, d.f. = 2, 29, $p = 0.53$).

The difference between BHR and NHR parasites was maintained after excluding six plant-pathogen associations ($\chi^2 = 5.01$, d.f. = 1, $p = 0.025$), two plant and plant-parasites ($\chi^2 = 5.32$, d.f. = 1, $p = 0.021$) and five vertebrate-parasite associations ($\chi^2 = 4.76$, d.f. = 1, $p = 0.029$; Table 2.2). The difference between NHR and BHR studies was no longer significant after excluding 13 tests on plants and herbivores or six tests on invertebrates and parasites (Table 2.2; $\chi^2 = 2.32$, d.f. = 1, $p = 0.127$ and $\chi^2 = 2.56$, d.f. = 1, $p = 0.109$, respectively). Studies using BHR parasites were more likely to produce null or negative outcomes than research based on NHR parasites; although this general result depended somewhat on the types of parasite–host associations chosen for retention in my analyses.

2.5 Discussion

Host range appears an important predictor of local adaptation. BHR parasites (78% of 32 tests) were more likely to show "no effect" or local maladaptation (68% of 25 tests), then NHR parasites (28.5% of 7 tests). Significant differences in effect sizes between studies based on BHR and NHR parasites were obvious overall, and after excluding three of five parasite–host associations. This general result was sensitive to loss of certain well-represented parasite–host associations, such as plants and their herbivorous insects (13 tests). However, IRT has limited ability to estimate effect size with such small samples (van der Linden & Hambleton 1997). The loss of significance when six tests based on invertebrate hosts and their parasites and pathogens were excluded is also not surprising since this reduced sample size for NHR tests from seven to five.

Host range is confounded, as it relates to the parasite–host associations under study (e.g., all plant parasites of plants and all vertebrate parasites have BHR). However, loss of these associations had no effect on the general result. Parsing data into phylogenetic groupings may reveal effects of evolutionary non-independence of certain taxonomic groups; but inability to properly estimate effects of small samples, coupled with deficiencies in the literature (e.g., lack of research on NHR parasites of vertebrates), prevent a thorough exploration of phylogenetic constraints on research outcomes. I also did not test whether respective BHR parasites have higher dispersal ability than NHR parasites (relative to their hosts). Further, I was unable to test whether geographical scale was similar between studies based on NHR versus BHR parasites, because

geographical scale would itself have to be scaled for relative dispersal abilities of different parasites and their hosts.

I contend that BHR parasites should show spatial evolutionary lags more than NHR parasites because BHR parasites undergo diffuse coevolution with multiple hosts (Futuyma & Slatkin 1983; Thompson 1994). I found that a large proportion of local adaptation studies was based on BHR parasites. Parasites with broad host ranges appear more the norm than rarity in nature. Progress will not be made in testing local adaptation and other coevolutionary hypotheses if these parasites continue to be treated as interacting with single species of hosts.

Future research should compare the ability of local BHR parasites on single species from two or more sites, where the suite of host species exploited differs between sites. Fitness performance on local hosts will likely depend on the suite of other potential hosts being exploited locally (further influenced by differences in local abundances). I predict that such parasites will be less likely to show high fitness on any given species locally, if confronted with a broad rather than a narrower host species pool.

Figure 2.1. The various types of transplant experiments used in testing for local parasite adaptation. In (a), two parasite populations (represented by black and white squares) are crossed to two host populations (represented by black and white circles). The colour of the square or circle denotes the location of the parasite or host population. Therefore in (a), there are two allopatric (experimental) and two sympatric (control) combinations of parasites infecting hosts. Experimental types (b), (c), and (d) are various permutations of type (a). Many more such combinations could be envisioned and have been tested (e.g., where more than two parasite populations are crossed with more than two host populations, which could be represented by more colours, but shown here as [::]).

(a) *(b)* *(c)* *(d)*

Table 2.1. Local adaptation studies (source) where information on the total number of known hosts (host range; from source study unless otherwise stated) and sample sizes (n^S and n^A , sympatric and allopatric respectively) are presented. I also present whether the study showed the parasite to be locally adapted (1), maladapted (-1), or showed no such outcome (0). Common garden experiments were either done (y) or not done (n). The type of experiment is also listed as coded in Figure 2.1 (*a*, *b*, *c* and *d*).

parasite species	host species	host range	n^S	n^A	study results	common garden	study design	source
pathogens and plants								
<i>Melampsora lini</i>	<i>Linum marginale</i>	min 5	16	20	1	y	<i>a</i> ^H	Burdon & Thompson (1995)
<i>Microbotryum violaceum</i>	<i>Silene dioica</i>	min 200 ^A	111	354	0	n	<i>a</i> ^H	Carlsson-Granér (1997)
	<i>S. latifolia</i>	min 200 ^A	14	14	-1	y	<i>a</i> ^H	Kaltz <i>et al.</i> (1999)
<i>Puccinia podophylli</i>	<i>Podophyllum peltatum</i>	1	102	204	0	n	<i>a</i> ^H	Parker (1989)
<i>Septoria tritici</i>	<i>Triticum aestivum</i>	min 10	30	40	1	y	<i>a</i> ^H	Ahmed <i>et al.</i> (1995)
<i>Synchytrium decipiens</i>	<i>Amphicarpaea bracteata</i>	1	13	11	1	y	<i>b</i>	Parker (1985)
herbivores and plants								
<i>Altica subplicata</i>	<i>Salix cordata</i>	6	9	9	0	y	<i>a</i>	Milanowski & Bach (1994)
<i>Apterothrips secticornis</i>	<i>Erigeron glaucus</i>	1	30	60	1	y	<i>a</i>	Karban (1989)
<i>Asphondylia borrichiae</i>	<i>Borrichia frutescens</i>	1 ^B	100	180	1	n	<i>a</i> ^H	Stiling & Rossi (1998)
<i>Blepharida rhois</i>	<i>Rhus glabra</i>	3	47	166	0	n	<i>a</i> ^H	Strauss (1997)
<i>Cryptococcus fagisuga</i>	<i>Fagus sylvatica</i>	3	5	15	1	n	<i>a</i> ^H	Wainhouse & Howell (1983)
<i>Daktulosphaira vitifoliae</i>	<i>Vitis arizonica</i>	7 ^C	6	66	0	y	<i>a</i> ^H	Kimberling & Price (1996)
<i>Matsucoccus acalyptus</i>	<i>Pinus edulis</i>	min 5	45	90	0	y	<i>a</i>	Cobb & Whitham (1998)
	<i>P. monophylla</i>	min 5	50	50	0	y	<i>c</i>	Unruh & Luck (1987)
<i>Nuculaspis californica</i>	<i>Pinus ponderosa</i>	11	20	80	0	y	<i>a</i>	Alstad (1998)

Table 2.2. The number of independent experiments (k), estimated effect sizes and 95% confidence intervals (CI) for tests on local adaptation for parasites with narrow host ranges (NHR) or broad host ranges (BHR).

host-parasite system	k	effect size	min CI	max CI
full data set ^A	32	-0.067	-0.198	0.063
NHR parasites	7	0.090	-0.080	0.260
BHR parasites	25	-0.259	-0.499	-0.039
excluding				
six plants and pathogens ^A				
NHR pathogens	5	0.234	-0.158	0.626
BHR pathogens	21	-0.314	-0.592	-0.036
13 plants and herbivores				
NHR herbivores	4	0.035	-0.167	0.236
BHR herbivores	15	-0.222	-0.484	0.040
two plants and plant-parasites ^A				
NHR plant-parasites	7	0.090	-0.080	0.260
BHR plant-parasites	23	-0.260	-0.505	-0.016
six invertebrates and parasites				
NHR parasites	5	0.088	-0.154	0.329
BHR parasites	21	-0.169	-0.369	-0.032
five vertebrates and parasites ^A				
NHR parasites	7	0.090	-0.080	0.260
BHR parasites	20	-0.205	-0.408	-0.002

^A NHR parasites significantly differed from BHR parasites (< 0.05).

CHAPTER 3

ON HOST TRACKING BY A MULTI-HOST ECTOPARASITE: IMPLICATIONS FOR LOCAL ADAPTATION STUDIES

3.1 Abstract

Relative local abundance of host species should determine the strength of selection from those hosts acting on parasite species locally. Parasites should encounter common hosts more often than less abundant hosts and should be selected to use common hosts more effectively. Parasites could do this by timing their infective stages with availability of common hosts and/or by evolving counter-adaptations to overcome defences of common hosts (because such hosts represent large targets of selection). I tested these premises of host species tracking for a parasitic water mite (*Limnochares americana*) and two of its dragonfly hosts (*Leucorrhinia frigida* and *Nannothemis bella*). Using field surveys, I confirmed that *L. frigida* was more abundant than *N. bella*, and that *L. americana* infests *L. frigida* more often and with higher intensity than it infests *N. bella*. These differences in host species use remained even after examining differences in habitat preference by males and females of both host species in relation to where mite larvae are active, and despite clear sex differences in mite infestation in *L. frigida*. Timing of hatching of mite clutches overlaps with abundance of young *L. frigida* hosts. Further, proportionately fewer mites died on *L. frigida* as compared

to *N. bella* hosts in nature. I also observed this pattern with experimental infestations, indicating that observed differences in suitability of the two species seen in nature could not be explained by differences in vigour of mites encountering the two host species. Taken collectively, my results support local tracking of a common host species by a multi-host ectoparasite; such tracking has implications for testing local adaptation theory in this and other parasite–host associations.

3.2 Introduction

Parasites are predicted to adapt locally to their hosts because parasites often have rapid generation times and/or larger population sizes than their hosts upon which selection can act (Hamilton 1980; see Chapters 1 and 2 and references therein). Additionally, if parasites have greater dispersal than their hosts, then incoming genetic variation can increase local genetic variation of parasites (Gandon 2002). Presumably, this greater genetic variation increases the probability of successful variants of parasites being favoured to exploit local hosts (Gandon *et al.* 1996; Gomulkiewicz *et al.* 2000; Nuismer *et al.* 2000). In some instances, rates of adaptation of parasites will be slowed when they have generation times or dispersal on par with their hosts (cf. Forbes *et al.* 1999), but parasites are generally thought to have faster rates of evolution.

Shorter generation times, greater numbers, and relative dispersal need not be the only factors, acting alone or in combination, affecting the rates of parasite adaptation and host counter-adaptation. Thompson (1994) argued that

coevolutionary potential of parasites should depend on the intensity of their interactions with other host species. Multi-host interactions are common in parasite–host systems, and many parasite species have a very wide range of host species locally, regionally or globally (Chapter 2; Woolhouse *et al.* 2001; hereafter parasites with multiple host species will be referred to as multi-host parasites). In some instances, parasites will likely enter into strong interactions with single host species when there is a clear primary host and one to several ancillary hosts (e.g., host species that are not primary targets of selection; Futuyma & Slatkin 1983). However in other instances, selection on parasites has the potential to differ in magnitude and direction because many host species are involved. As such, the intensity of reciprocal selection with any particular host species may be dampened (known as diffuse coevolution; Agrawal 2000a; Stinchcombe & Rausher 2001). This dampening should result in a slower rate of evolutionary change for parasites on their (primary) hosts, similar to Whitlock's argument (1996) on the affects of niche breadth on rates of evolutionary change.

Although evolutionary rates in multi-host parasites may be slower than in more specialised species, one may expect multi-host parasites to 'track' their most common local host species. Because more individual parasites encounter and exploit the more common host species, this should increase the likelihood of successful variants arising that are better able to exploit this host species. This argument is similar to those outlined where increased genetic variability of parasites and sheer numbers of parasites influence the likelihood of local adaptation of 'specialised' parasites. However, tracking in local host species

attempts to explain fitness variation of parasites on different host species. Fitness of parasites with multiple hosts does vary across host species (Mouahid & Théron 1987; Forbes *et al.* 2002). Making sense of this variation is a troubling problem for both evolutionary biologists and parasitologists.

Local tracking of abundant host species is not the same as local parasite adaptation, yet the former has clear implications for testing the latter. To begin with, parasite populations are exposed locally to species that do not encompass their theoretical maximum host range (Rohde 1994; Poulin 1998). As such, hosts that are common at one locale, may be less of a target of selection (or even a non-target) at another locale, thereby making results from reciprocal transplant experiments (which are designed to detect local parasite adaptation) difficult to interpret (see Chapter 2). There is a consensus that predictions of local adaptation theory should be tested through reciprocal comparisons involving multiple parasite and host populations, to determine whether local adaptation exists and, if so, at what scale (cf. Ebert 1994; Gandon 1998). However, if researchers use populations where the host species of interest is not the primary target of selection, then parasite fitness on local versus non-local hosts may not be predicted easily.

Patterns consistent with local tracking of host species include ones where parasites have higher fitness on more abundant hosts, and/or have timed infective stages to take advantage of locally abundant hosts. The degree to which host tracking occurs is not well studied, nor are its implications for testing local adaptation theory. Importantly, the same processes that should give rise to local

tracking of a host species can potentially result in failure to find local parasite adaptation using reciprocal transplant experiments. The likelihood of this failure will likely depend on the particular host species that is chosen for study, within suites of host species available at two or more sites (see Chapter 2).

In this study, I tested for host species tracking, using two dragonfly hosts parasitized by an ectoparasitic mite species. I first determined whether the two host species differed in local abundance and whether mites more often exploited one of those species. I also tested whether the parasite timed its phenology to take advantage of the more abundant species and whether the more abundant host species also was more susceptible to the parasite, using both collections and experimental infestations. I included a comparison of habitat selection by males and females of both host species (relative to where mite larvae were active) to elucidate any potential causes of within or among species variation in infestation. My study shows that degree of host use and parasite fitness depends on the host species under consideration, even after accounting for variation in infestation within one species (attributable in part to differential habitat use by males and females). Genetic constraints may restrict patterns of host use (Agrawal 2000*b*; but see Via & Hawthorne 2002), but this is not the case in my study (see below). Finally, I argue that results of my study have implications for testing predictions of local adaptation theory.

3.3 Materials and Methods

3.3 a) *Study association*

I studied the ectoparasitic water mite, *Limnochares americana* Lundblad and two of its dragonfly hosts, *Leucorrhinia frigida* Hagen and *Nannothemis bella* (Uhler). These two hosts are common to sphagnum bog ponds in northeastern North America, with *N. bella* being almost exclusive to these bogs (Walker & Corbet 1975). *Limnochares americana* exploits a minimum of 25 species of hosts including both damselflies (suborder Zygoptera) and dragonflies (suborder Anisoptera) (Conroy & Kuhn 1977; Cook 1991), indicating that genetic constraints on use of odonate hosts may not be strong. Work 11 years ago at my study site showed that *L. frigida* was more often used as a host than *N. bella* (Cook 1991), but abundance data on the two species was not considered explicitly.

Several aspects of the natural history of this water mite–dragonfly system are relevant to my study. Female *L. americana* lay clutches on submerged stems of emergent vegetation (Smith & Oliver 1986). Upon hatching, mite larvae swim to the surface of the water and crawl up on this emergent vegetation. When an adult dragonfly perches, larval mites climb on it, but may be removed by grooming (Léonard *et al.* 1999). Otherwise, larvae will attach to the host by inserting their chelicerae through the host's cuticle (Smith 1988), presumably if the host is deemed suitable. Following attachment, the larval mite secretes mucopolysaccharides into the host's haemocoel to form a feeding tube (Smith 1988). If attachment and feeding tube formation is successful (e.g., not countered by host melanotic encapsulation; Gillespie & Kanost 1997), the larva begins to

feed and engorge. Once fully engorged, the larval mite drops off the host (when the host is over water), and completes its life cycle as an aquatic predator (details in Smith 1988).

3.3 b) Do dragonfly hosts differ in abundance or exploitation by water mites?

I first tested whether *L. frigida* and *N. bella* differed in abundance, by surveying dragonflies during 25 trips to my study site between 21 June and 18 August, 2001. My study site was Hebert's bog located 10 km from the Queen's University Biological Station near Chaffey's Locks, Ontario Canada (44° 35' N; 76° 19' W). These censuses occurred at ca. 10:30 EDT and ended after one hour. Dragonflies were netted at the edge of the bog where the sphagnum mat meets open water. Gender and approximate age were recorded for each dragonfly (cf. Walker 1953); where teneral or newly emerged adults had soft, shiny wings, with a soft abdomen with little pigmentation on the exoskeleton. Mature adults had rigid, dull wings with full body pigmentation. I assumed that each species was similarly visible and netted with equal success. As a first approximation, I compared species abundance with the expectation that each species accounted for 50% of captures, using Chi-square tests (Zar 1984).

I next compared prevalence and intensity of mite infestation (following Bush *et al.* 1997) and overall numbers of mites on 147 male and 23 female *L. frigida* and 66 male and 22 female *N. bella*. Each dragonfly was marked, then released on site, after dotting wings with non-toxic Sanford Sharpie® pens (Sanford Corporation, Bellwood, U.S.A.). Marked dragonflies were not re-sampled.

Acquisition of new *L. americana* larvae occurs throughout the entire life of the adult dragonfly, such that larvae in several stages of engorgement could be present on a single host. I counted engorging or engorged mites and dead mites on each dragonfly using a 20x loupe. Engorging or engorged mites had their legs partially or entirely obscured by their swollen bodies, whereas dead mites were flat and silver in colour (see Léonard *et al.* 1999). I also recorded crawling or phoretic mites. *Limnochares americana* larvae have an 'aerial' crawling phase when they search for potential hosts. These larvae are red and weakly sclerotised. The number of crawling or phoretic mites (hereafter phoretic mites) could be used as an index of 'attempts' at parasitism. I tested whether there were more phoretic mites on either host species, while testing whether dragonfly species and sexes differed in parasitism by *L. americana*. Chi-square tests of association were used for prevalence data (Zar 1984), whereas Wilcoxon two-group tests were used to test for differences in intensity of mite infestation or in numbers of phoretic mites. Because some of the Chi-square tests violated the assumption of $< \frac{1}{4}$ cells with expected frequencies < 5 , I also calculated 95% confidence intervals around proportions (following Zar 1984) to illustrate further where differences in prevalence existed. Finally, all of these tests were based on individuals collected at the bog's edge during the surveys described above.

3.3 c) *Habitat selection by males and females of both species* ***and degree of parasitism***

Previous work at my site showed that male *L. frigida* and male *N. bella* carried more larval *L. americana* than did conspecific females (Cook 1991), although the results were significant only for *L. frigida*. As indicated above, males of both species were more often caught than females at the bog edge. One obvious explanation is that many males of both species perch and hold territories at the edge of the bog (Hilder & Colgan 1985; Lee & McGinn 1986; Smith & Cook 1991), whereas females tend to visit the bog edge only for mating and egg laying. The bog edge is where mite clutches are laid and where larval mites are active (see below). I was interested in whether one species may have used edge habitat more than another. In this second comparison of host use patterns, relative to habitat use by hosts and larval mites, I confined collections to two sampling days two days apart; thus, controlling for any seasonal changes in mite abundance. I first did a two-hour survey on July 14, 2001 in the morning 2-4 meters from the bog edge. A second survey was done 18 meters from the edge in the afternoon. This sampling perimeter was established by placing flagging rods 18 meters away from corresponding flagging rods set along the bog edge (distance was measured using a Bushnell Pro 400 laser rangefinder). On July 16, I reversed the order of sampling of the habitats, sampling 18 meters from the edge in the morning and at the bog edge later in the early afternoon. Dragonflies were collected, marked, and had their larval mites counted, as described above. I did not resample individuals that were marked.

I used a three-way log-linear analysis to identify any associations between sex, habitat use (measured coarsely as where individuals were caught) and infestation by *L. americana* larvae (no mites versus one or more attached mites). I did this analysis by including these variables as independent factors in a general log-linear model. My interpretations of results followed from Knoke & Burke (1980) and were somewhat different from interpretations following usual statistical models. Here, a significant overall model is one with $p < 0.05$ which suggests non-independence. If factors were interdependent, then I proceeded by systematically screening significance of interactions among main effects, and significance of the main effects themselves, until finding the simplest model that best described my field observations. Each log-linear model generates a particular set of expected frequencies (analogous to a goodness of fit Chi-square; Zar 1984). I can select the model that best fits patterns in my data, by associating these expected frequencies with my observed frequencies (but here, a non-significant comparison between expected and observed, with $p > 0.1$, indicates that the model explains the observed data). If, for example, inclusion of location relative to the bog edge generates a non-significant model, then inclusion of location is necessary to get 'fit' between observed and expected frequencies and thus is a contributor to patterns of infestation.

3.3 d) Phenology of dragonflies and egg laying and hatching of mites

I next determined whether *N. bella* and *L. frigida* differ in timing of emergence, by sampling exuviae of both species, during 17 of the 25 trips to

Hebert's Bog. Exuviae were collected from emergent vegetation in four 2-by-1 m quadrats located along the bog edge. All exuviae were stored in 75 ml plastic vials with bog water for transport (hydrating exuviae was necessary as many were brittle). Once at the laboratory, vials were stored in a Fisher incubator at ca. 5 °C until identification. For identification, exuviae were viewed using a dissection microscope (10x; Nikon SMZ-1). Exuviae were identified to species (following larvae keys in Walker & Corbet 1975) only if they were from larval to adult moults; where wing sheaths reached at least the fourth abdominal segment (Corbet 1999), and if the lateral spines were intact on the last four abdominal segments (necessary for identification).

I also inspected emergent vegetation for mite clutches in the same four 2-by-1 m quadrats on 15 trips every third day between June 23 and August 10, 2001. I recorded days on which clusters of *L. americana* clutches appeared. Once found, emergent stems acting as oviposition sites were labelled with numbered flagging tape. On each visit, position of each clutch was noted and each clutch was scored as having either hatched (semi-translucent/white in colour, no larvae visible inside eggs) or as not having hatched (red, larval mites still present within the clutch). Because dates of clutch appearance and hatching were non-normal, I compared them to median dates of collection of exuviae using Wilcoxon tests. As visits were made every third day, hatching dates were approximated as a minimum date of hatch (previous date of visit if clutch was hatched by date of current visit) and also as a maximum date of hatch (date of current visit if clutch had hatched). I did this because I could have scored a clutch

as not having hatched on a previous visit, but it had, in fact, hatched later that same day, after surveys were conducted. Actual median dates of hatch were between these two extremes.

3.3 e) *Fitness of mites on different dragonfly hosts*

Finally, I examined relative suitability of each host species for *L. americana*, using experimental infestations. These tests complemented my earlier surveys in which numbers of dead mites versus live, attached mites were compared between host species. The earlier surveys would indicate whether one host species was more likely to have dead mites, but this could be due either to a difference in host suitability or to differences in vigour of mites attending the two host species. Stated another way, I had to control for the possibility that mites attending one host species were nearing exhaustion, after searching for a more suitable host. Such mites may have been relatively easy to defend against. For this test, 62 adult female mites were collected between July 4 and July 6, 2001 at Herbert's bog and placed in water filled dram-vials containing short wooden dowels as a perch and an oviposition site. Vials were placed in an incubator (24.5 ± 1 °C; Blue M, Electric Company, Blue Island, U.S.A.) until clutches were laid. Twelve females laid clutches (after 9.2 ± 0.8 days; mean $\pm$ SE) of which ten hatched after 5.4 ± 0.9 days, and averaged 61.8 ± 10.9 mite larvae per clutch. These phoretic larvae were transferred onto experimental hosts on the day of their hatching.

For experimental transfers, *L. frigida* and *N. bella* adults were collected from Hebert's bog on 10 trips from 15 to 27 July, 2001. I collected individuals with few

or no mites. Smith and Cook (1991) showed that number of larval mites is indicative of age of male *L. frigida* hosts (old males have more mites). I collected relatively young adults, but did not use teneral dragonflies because they were too fragile to be held by their wings during experimental infestations, and because they quickly desiccate under laboratory conditions. In total, 22 male and 8 female *L. frigida*, and 20 male and 13 female *N. bella* were collected. I first counted phoretic, dead, and attached mites to test whether initial mite numbers differed between species (any mites on these hosts prior to experimentation were not included in subsequent analyses). Any non-experimental phoretic mites were first removed with a fine paintbrush, and individual dragonflies were placed in plastic cups containing 2 ml of water beneath a window mesh screen, and with a wooden dowel serving as a perch (following Leung & Forbes 1997). Each cup was covered with aluminium foil and placed in an incubator for a minimum of two hours (24.5 ± 1 °C; Blue M, Electric Company, Blue Island, U.S.A.).

Individual dragonflies of both species were challenged with experimental phoretic larvae on the day of their collection and the day of mite hatching (depending on the experimental subjects and the mite clutch used in experiments). To challenge dragonflies, I held each dragonfly by its wings, and by positioning the venter of its thorax near the tip of the wooden dowel (within the dram vial), I waited until mite larvae climbed onto it (following Léonard *et al.* 1999). I controlled as much as possible for numbers of experimental larvae on each host by using a fine paintbrush (resulting in 6.6 ± 0.2 mites per dragonfly; mean $\pm$ SE). Individuals were then returned to the incubator for three days, removed at regular intervals for

feeding, after which time mite engorgement success was scored (as above). Feeding was necessary because Léonard *et al.* (1999) found that *L. americana* larvae may delay engorgement if hosts are deprived of food. I manually fed experimental subjects each day with chironomids (midges) and/or trichopterans (caddisflies) collected from UV lights at night.

As noted above, I collected few dragonfly females for experimentation because I found few females of the appropriate age. I pooled results between sexes in order to make general inferences between species on their ability to defend against *L. americana* larvae. This procedure is appropriate because I found statistically indistinguishable numbers of dead mites between sexes within each species (see Results). I analysed my data using Shirley's (1981) non-parametric, one-way ANCOVA as the assumption of normality for the dependent variable(s) was violated (Zar 1984). Non-parametric ANCOVA is performed similarly as normal ANCOVA, but with average ranks of the dependent variables rather than actual observations. Test results are then compared to Chi-square distributions instead of *F*-distributions (see Shirley 1981). My first test was to examine differences in mite attachment between host species, controlling for the number of experimental mites used and for the trial date. I thought it important to control for trial date statistically because there was some expertise developed in conducting these experiments and trial date might reflect better handling of dragonflies and mites. The adjusted mean ranks of this model represent the variation in mite attachment corrected by these two factors. I next compared differences in the number of failed attempts to parasitize host species by

comparing the number of dead mites between species, again using non-parametric ANCOVA, corrected for the number of attached mites as a covariate. I also conducted tests to elucidate if biases may have existed from the inclusion of individuals with extreme phenotypic characteristics; but these results are not reported herein in this Chapter (see Appendix C for similar results after excluding individuals with extreme phenotypes).

3.4 Results

3.4 a) *Host abundance and water mite exploitation*

Adult *L. frigida* and *N. bella* dragonflies differed in total seasonal abundance at my site; with the former dragonfly being most abundant (frequency $\chi^2 = 26.52$, d.f. = 1, $p < 0.001$). Prevalence, abundance and intensity of attached mite larvae also differed significantly between dragonfly species (Table 3.1), with *L. frigida* having the greatest *L. americana* infestation (Table 3.1). There was within species variation of mite infestation based on the first survey: male *L. frigida* had greater parasitism than females (Table 3.1; cf. Smith & Cook 1991). In contrast, *N. bella* did not show sex biased infestation; although, males had higher larval abundances than females (Table 3.1). In total, only 2.7% of 1527 *L. americana* mites were found on *N. bella*. Based on field surveys, *N. bella* had successfully defended against more mites (Table 3.2). There were no differences in likelihood of one or more dead mites between sexes (in either species), nor were there differences in the median number of dead mites between males and females of either species

(Table 3.2). Finally, *L. frigida* males had more phoretic mites than conspecific females, but this was not the case for *N. bella* (Table 3.3).

3.4 b) *Habitat selection by males and females of both species and degree of parasitism*

I found that each species was equally represented at both outer and edge regions of the bog: 44% and 56% of *L. frigida*, and 47% and 53% of *N. bella* were found at outer and edge regions, respectively ($\chi^2 = 0.1$, d.f. = 1, $p = 0.75$). Again, more *L. frigida* ($N = 92$) were captured than *N. bella* dragonflies ($N = 55$), similar to the first survey ($\chi^2 = 9.4$, d.f. = 1, $p = 0.002$; see Chapter 3.4 a)). There was no differential use of habitats by species and, thus, this could not explain species differences in mite parasitism.

One of my log-linear analyses revealed significant non-independence between sex, the sample location relative to the edge of the bog and mite infestation status for *N. bella* dragonflies ($G_{(s, l, i)}^2 = 23.3$, d.f. = 4, $p < 0.001$; where the factors refer to 's' = sex, 'l' = location and 'i' = infestation). However, the best final model accounting for the variation between these factors showed that each sex preferred different regions of the sphagnum bog, but prevalence of infestation was independent, and could not be predicted by sex and/or location relative to the edge ($G_{(s \times l, i)}^2 = 0.2$, d.f. = 1, $p = 0.648$). Specifically, 69% of male *N. bella* were captured at the edge, whereas all females were captured distant to the bog edge. In *L. frigida* by comparison, there was significant non-independence between sex, location and prevalence of infestation ($G_{(s, l, i)}^2 = 34.5$, d.f. = 4, $p < 0.001$). For *L.*

frigida, the best fit model showed that all these factors were inter-related, and that their associations contributed to observed patterns at my bog ($G_{(s \times l, i \times l, i \times s)}^2 = 3.9$, d.f. = 3, $p = 0.262$). For instance, 19% of females and 64% of males were found near the bog edge, but at the edge 79% and 33% of these males and females were infested with at least one mite. Here, sexes were exposed to mites differentially because of habitat choice, and this was reflected in their different degrees of *L. americana* parasitism overall. As such, sex differences in habitat use (and perhaps sex related differences in time spent perching at the edge) could predict *L. americana* infestations on *L. frigida*.

3.4 c) Phenology of dragonflies and egg laying and hatching of mites

Water mite females are expected to lay clutches in synchrony with their hosts, to maximize exposure of their larvae with these hosts (e.g., Rolff 2000). Median emergence time between *N. bella* and *L. frigida* dragonflies differed (Wilcoxon $Z = -8.29$, $p < 0.001$); more *N. bella* final-instar exuviae were found earlier in the season than those of *L. frigida* (Table 3.4). Appearance of *L. americana* clutches did not correspond with either dragonfly emergence (Table 3.4; *L. frigida*: Wilcoxon $Z = -2.64$, $p < 0.001$; *N. bella*: Wilcoxon $Z = 3.79$, $p = 0.008$). However, time-of-hatching was generally 12 days later (inter-quartile range = 8-19, total range = 2-26, $N = 23$). There was significant overlap between the time of hatching of mite larvae and *L. frigida* emergence (Wilcoxon $Z = 0.1287$, $p = 0.129$), but not with *N. bella* (Wilcoxon $Z = 5.71$, $p < 0.001$). However, the latter results were based on the maximum median time of eggs to hatch (see

rationale in Methods 3.3 d)). Minimum hatching time was calculated as hatching time minus the number of days since the last observation period. With these corrected data, minimum median hatching time again overlapped with the median emergence of *L. frigida* exuviae (Wilcoxon $Z = 0.37$, $p < 0.707$), but not with *N. bella* emergence (Wilcoxon $Z = 5.05$, $p < 0.001$). Thus, approximate hatching periods of *L. americana* larvae in nature correspond more with *L. frigida* emergence than with *N. bella* emergence.

3.4 d) Fitness of mites on dragonfly hosts

Experimental mites attached equally to each dragonfly species; even after correcting for the number of mites used in trials and the timing of trial (two significant covariates; Table 3.5; see Table 3.7 for mean attachments). However, larval *L. americana* attaching to *N. bella* suffered greater mortality than those attaching to *L. frigida* dragonflies (after accounting for the total number of live and attached mites; Tables 3.6 and 3.7).

3.4 e) Summary of salient findings

Leucorrhinia frigida was more abundant than *N. bella* and had higher numbers of phoretic and attached mites, but fewer dead mites, than *N. bella*. Species differences in host use remain even after accounting for differential habitat use within species (in relation to where mite larvae are active), and despite the sex differences in infestation in *L. frigida* that result from differential habitat use and/or differences in perching behaviour. More mites on *L. frigida*, the common host, is

expected to influence the likelihood of successful variants being favoured locally to exploit this species better. *Leucorrhinia frigida* accounts for over 97% of the *L. americana* mites on these two species at my study site. I further found that *L. americana* has infective larvae that overlap temporally with emergence of many young *L. frigida* adults and that mites attending this species are more likely to survive than mites attending *N. bella* dragonflies. This greater suitability of *L. frigida* dragonflies is apparent after controlling for relative age of dragonflies and for mite vigour.

3.5 Discussion

The factors affecting successful exploitation of different host species are not clear. Some hosts may accept parasites rather than pay costs of resisting (Forbes 1993; Davies *et al.* 1996). Alternatively, selection on the parasite may result in its ability to track and exploit a common species, more so than a rare species. I found that a multi-host parasite uses the most common host species at my site much more often, even after accounting for sex differences in infestation of the common species. This result appears due to timing of infective stages of the mite (i.e. local tracking). Further, there is a greater fitness cost for mites exploiting the less abundant species. I suggest that local abundance of host species should be explored further as a predictor of host use and parasite fitness. However, host abundance should not be examined in isolation of other factors relating to the intensity of interactions (Thompson 1994), such as age of association or genetic

constraints on host use which could explain the contrary finding by Singer *et al.* (1989).

My study represents a detailed case study. However, the results are consistent with another water mite dragonfly association. Forbes *et al.* (1999) showed that the more abundant species of two *Sympetrum* dragonflies carried a much greater proportion of *Arrenurus planus* water mites (> 92%), than did a rare host species. The latter host species was entirely resistant to mite attachment. They concluded that there should be no differences in cost of parasitism to these similarly sized dragonflies and thus no reason to expect greater resistance in one species than another. In fact, prevalence and intensity of mite infestations were equal between these two species, despite many fewer hosts of one species carrying many fewer mites overall. Here again, the mite appeared to time its activity with the emergence period of the more abundant species (Forbes *et al.* 2002). In the present study, *N. bella* is a much smaller dragonfly and may be expected to resist water mites more so than *L. frigida*. Water mite parasitism has been associated with significant fitness costs in odonates in some studies, but not in others; these include reduced flight performances, reduced mating success and reduced survivorship (see Léonard *et al.* 1999 and references therein). However, the numbers of mites on *L. frigida* far exceed levels where some resistance is expected (in other words, the volume of engorging mites relative to the volume of the dragonfly is higher for *L. frigida* than for *N. bella*). The relationship between body size and costs associated with parasitism across species remains equivocal (see Poulin 1995; Poulin & Rhode 1997; Morand & Poulin 1998). Another

explanation is that *L. americana* is not optimised to infest *N. bella*, such that *N. bella* can maintain some degree of resistance without having to constantly reshape its defences against this parasite. Following reasoning in Nee *et al.* (1991), *N. bella* may 'hide' in the background of the major interaction between *L. frigida* and *L. americana*; but see Singer (1983) for a counter-example of a less abundant host being more exploited.

My study has implications for how to approach and test local adaptation theory. First, researchers need to assess fitness of parasites on non-target hosts (such as *N. bella* dragonflies at Hebert's bog) to the fitness of this parasite where such host species are the target of selection (in other locale, where *N. bella* is more abundant, or *L. frigida* is less abundant). In fact, *L. americana* does not even use these two species in other parts of its range (B. P. Smith, *personal communication*). Such tests will enable researchers to produce a conceptual framework for predicting where local adaptation should occur and where it may be absent. Here, the premise is that selection favouring adaptations for one host species at one particular site results in a trade-off in the use of another (due to genetic correlation; *sensu* Via & Hawthorne 2002). Comparing mites from different locales (or offspring from crosses) could help elucidate such genetic trade-offs by testing mites and offspring against different host species backgrounds. Progress would be made also by comparing the fitness of parasites on target host species (such as *L. frigida*), but in locations where these parasites have a larger suite of potential hosts. Here, I expect lower fitness on the host species of interest (associated with less overlap of emergence or activity times). Such comparisons

of host and parasite fitness between sites differing in suites of suitable hosts will allow researchers to understand better conditions favouring local adaptation versus local maladaptation.

TABLE 3.1. Number of dragonflies (by species and sex) that were uninfested (N_u) versus infested (N_i) by one or more *L. americana* larvae. Prevalence of infestation and confidence interval (CI; following Zar 1984), median abundance and intensity of infestation (M) are provided with associated inter-quartile ranges and full ranges (IQR and R, respectively). Chi-squares (d.f. = 1) and associated p -values ($\chi^2(p)$) are presented for comparisons of prevalence between males and females, and separately for each species. Wilcoxon tests (Z) were used to compare median abundance and intensities, between sexes. Combined values for males and females of each species are also presented (all).

species	sex	N_u	N_i	prevalence ^A			abundance ^B			intensity ^{C,D}		
				%	CI	$\chi^2(p)$	M	IQR:R	Z (p)	M	IQR:R	Z (p)
<i>Leucorrhinia frigida</i>	male	11	136	92.5	4.3	45.2	8	4-14:0-47	-6.39	9	5-14:1-47	-4.07
	female	14	9	60.9	19.9	(<0.001)	0	0-2:0-5	(<0.001)	2	1-3.5:1-5	(<0.001)
	all	25	145	85.3	5.3		6	2-13:0-47		8	4-14:1-47	
<i>Nannothemis bella</i>	male	47	19	28.8	10.9	3.5	0	0-1:0-4	-1.96	1	1-3:1-4	-1.14
	female	20	2	9.1	12.0	(0.061)	0	0-0:0-1	(0.050)	1	1-1:1-1	(0.256)
	all	67	21	23.9	8.9		0	0-0:0-4		1	1-3:1-4	

^A between host species: $\chi^2 = 95.4$, d.f. = 1, $p < 0.001$

^B between host species: $Z = -10.5$, $p < 0.001$

^C between host species: $Z = -6.04$, $p < 0.001$

^D only dragonflies with one or more mites were included in analysis

TABLE 3.2. Number of dragonflies (by species and gender) that were infested with one or more *L. americana* larvae (N_i), versus dragonflies infested, but had one or more dead larvae (N_k). The percentage of individuals that successfully resisted one or more mites (%) is simply $N_k / (N_k + N_i)$; the associated confidence intervals (CI) for these percentages were calculated following Zar (1984). The median number of dead mites (M), and associated inter-quartile and full ranges of dead mites (IQR and R, respectively) are also provided. Chi-square test statistics (d.f. = 1) and associated p -values ($\chi^2 (p)$) are presented for percentage of males or females with one or more dead mites, and separately for each species. Wilcoxon tests (Z) were used for the comparison of median number of dead mites, and between sexes of *N. bella* dragonflies (too few data were available to analyse sex differences in *L. frigida*). Combined values for males and females of each species are also presented (all).

species	sex	N_i	N_k	individuals with dead mites ^A			number of dead mites per individual ^{B, C}		
				%	CI	$\chi^2 (p)$	M	IQR:R	Z (p)
<i>Leucorrhinia frigida</i>	male	131	5	3.7	3.2	0.343	1	1-2:1-2	
	female	9	0	0.0	0.0	(0.558)	0	0-0:0-0	
	all	140	5	3.5	3.0		1	1-2:1-2	
<i>Nannothemis bella</i>	male	10	9	47.4	22.5	0.005	1	1-2.5:1-4	-0.59
	female	1	1	50.0	69.3	(0.944)	1	1-1:1-1	(0.555)
	all	11	10	47.6	21.4		1	1-2.25:1-4	

^A between host species: $\chi^2 = 43.54$, d.f. = 1, $p < 0.001$

^B between host species: $Z = -0.21$, $p = 0.834$

^C excluding dragonflies with no dead mites

TABLE 3.3. The number of dragonflies (by species and gender) with crawling or phoretic *L. americana* mites (N_p) versus those that did not have any larvae (N_n). Percentage of individuals with phoretic larvae (%) is simply $N_p / (N_n + N_p)$; confidence intervals for these percentages (CI) were calculated following Zar (1984). Median number of phoretic larvae (M), and associated inter-quartile and full ranges (IQR and R, respectively) are also provided. Chi-square test statistics (d.f. = 1) and associated p -values ($\chi^2(p)$) are presented for comparisons of proportions of males and females with phoretic mites, and separately for each species. Wilcoxon test statistics (Z) were used for the comparison of median number of phoretic mites, between sexes. Combined values for males and females of each species are also presented (all).

species	sex	N_p	N_n	individuals with phoretic mite larvae ^A			number of phoretic larvae per individual ^{B, C}		
				%	CI	$\chi^2(p)$	M	IQR:R	Z(p)
<i>Leucorrhinia frigida</i>	male	70	77	47.6	8.1	8.1	2	1-3:1-7	-1.39
	female	4	19	17.4	15.5	(0.007)	1	1-1.75:1-2	(0.163)
	all	74	96	43.5	7.5		2	1-3:1-7	
<i>Nannothemis bella</i>	male	13	53	19.7	9.6	3.48	1	1-2:1-2	-0.47
	female	1	21	4.5	8.7	(0.062)	1	1-1:1-1	(0.635)
	all	14	74	15.9	7.6		1	1-2:1-2	

^A between host species: $\chi^2 = 21.2$, d.f. = 1, $p < 0.001$

^B between host species: $Z = -2.22$, $p = 0.027$

^C excluding dragonflies that did not have phoretic mite larvae

TABLE 3.4. Emergence dates of dragonflies based on surveys of exuviae, and appearance and hatching times of *L. americana* clutches. *N* refers to the total number of exuviae or clutches found during a 50-day survey. The median emergence date is also reported (M; with day one corresponding to the 171st Julian day of 2001). Additionally the inter-quartile and full range of dates (IQR and R, respectively) are also provided. See Chapters 3.3 d) and 3.4 c) for descriptions of minimum and maximum hatching time and for tests examining differences between emergence periods.

species	<i>N</i>	<i>M</i>	IQR	R
parasite				
<i>Limnochares americana</i>				
appearance of clutches	36	15	3 - 25	3 - 51
minimum hatching time	30	22.5	15 - 39	6 - 47
maximum hatching time	30	30.5	21 - 49	12 - 55
dragonfly host exuviae				
<i>Leucorrhinia frigida</i>	201	28	10 - 34	1 - 51
<i>Nannothemis bella</i>	182	4.5	1 - 20	1 - 47

TABLE 3.5. Results of the non-parametric ANCOVA (Shirley 1981) on the number of *L. americana* mite larvae that attached to *L. frigida* and *N. bella* dragonflies after experimental exposure. The number of phoretic mites used to challenge hosts (number challenged) and the timing of trial (timing) were used as covariates (cov). Adjusted means for numbers of attached mites between species, after correction by ANCOVA, are reported in Table 3.7.

tests	d.f.	SS	MS	χ^2	p
parallelism between slopes					
species x timing	1	387.56	387.56	1.186	0.276
species x number challenged	1	568.37	568.37	1.739	0.187
species x timing x number challenged	1	570.84	570.84	1.747	0.186
Total	62	20257.50	326.73		
ANCOVA					
timing (cov)	1	2405.61	2405.61	7.362	0.007
number challenged (cov)	1	1182.07	1182.07	3.618	0.057
species	1	520.36	520.36	1.593	0.206
Total	62	20257.50	326.73		

TABLE 3.6. Results of the non-parametric ANCOVA (Shirley 1981) on the number of dead *L. americana* larvae found on *L. frigida* and *N. bella* after experimental exposure, and after using the number of attached mites as a covariate (cov). Adjusted means for number of dead mites found between species, after correction by ANCOVA, are reported in Table 3.7.

tests	d.f.	SS	MS	χ^2	p
parallelism between slopes					
species x					
number of attached mites	1	782.92	782.92	3.718	0.054
Total	62	13055.00	210.56		
ANCOVA					
number of attached mites (cov)	1	432.19	432.19	2.053	0.152
species	1	3774.35	3774.35	17.920	0.001
Total	62	13055.00	210.56		

TABLE 3.7. Mean values with standard errors (SE) for the number of attached mites and the number that died following attachment on each dragonfly host species after experimental exposure with phoretic *L. americana* larvae. Also presented are the average ranks and adjustment after non-parametric ANCOVA (Shirley 1981).

species	mean	SE	unadjusted rank		adjusted rank	
			mean	SE	mean	SE
attached mites ^A						
<i>Leucorrhinia frigida</i>	3.97	0.43	31.68	3.85	31.99	3.02
<i>Nannothemis bella</i>	4.09	0.26	32.29	2.64	32.01	2.87
dead mites ^{B, C}						
<i>Leucorrhinia frigida</i>	0.03	0.03	23.97	0.97	23.96	2.16
<i>Nannothemis bella</i>	0.70	0.14	39.30	2.85	39.55	2.05

^A adjusted after controlling for timing of trial and the number of mites challenged

^B adjusted after controlling for number of attached mites

^C test for species differences, $p < 0.05$, see Table 3.6

CHAPTER 4

SUMMARY AND PROSPECTUS

In Chapter 2, I provided the first general test of whether local adaptation was affected by the degree of host use by parasites, using analyses based on item response theory. I showed that research based on parasites with broad host ranges was less likely to support local adaptation than studies using parasites with principally one host, most likely because such parasites enter into diffuse evolutionary interactions with these hosts (Futuyma & Slatkin 1983). My study further indicates deficiencies in the published literature on local adaptation, which have slowed progress in understanding this phenomenon on other theoretical fronts (e.g., influence of host species range as opposed to differential gene flow; see below and see Chapter 1). Furthermore, local adaptation research appears to have been biased by studies using parasites with multiple host species. Progress will not be made unless more research is performed on single-host parasites, or if design of experiments account for parasites that use multiple host species.

In Chapter 3, I found that a multi-host ectoparasite could track a locally common host species, by first timing infective stages to match those of the common host, and by having greater success in circumventing this hosts defences compared to a less common host's defences. These results correspond with coevolutionary predictions that the intensity of interactions with common or

abundant host species, should influence the degree in which these hosts become targets of selection. Further, my study shows that ranking hosts based only on host preferences observed from field data, can be misleading, especially when spatial and temporal availability of host species and/or host sexes may affect the observed distributions of parasites.

I would like to conclude with emphasis on the criteria for ascertaining local adaptation in parasites with multiple hosts. First, I would like to outline the broad predictions of local adaptation based on coevolutionary theories with spatial structure (referred to as the geographic mosaic theory for coevolution; see Thompson 1997, 1999), and then indicate how my predictions based on multi-host parasites differ from these. Briefly, Gandon *et al.* (1996) theorised that, if under meta-population conditions, parasites (or hosts) migrate more than hosts (or parasites), then these parasites (or hosts) should be locally adapted. However, if both parasite and host migrate equally then local adaptation should not occur. As such, the source driving geographic mosaics of coevolving species are differential migration events between parasites and hosts.

Augmenting local parasite adaptation to larger spatial scales should not, however, exclude factors that make populations, or particular locations, distinct (e.g., factors that shape host races of parasites; Jaenike 1981). My predictions of local adaptation includes geographical variation as a product of differences in host species distributions and abundance. It is known that geographically the distributions of many parasite species overlap with distributions of few to many host species (Rohde 1994). Changes in abundance and distribution of secondary

species can alter patterns of selection on primary host species; where the presence or absence of particular host species can alter or diffuse responses of selection by parasites (see Stinchcombe & Rausher 2002). Thus variation in host exposure, much like effects of migration events, can also drive evolutionary forces in generating geographical mosaics of coevolution.

One further point must be made. If experimentation is approached without accounting for host use, then predictions based on migration theory may be inadequate or misleading (see Chapter 3). However, experiments designed to test local adaptation in the face of multiple hosts use may provide a better framework to observe effects and consequences of local selection (see Table 4.1 for revised experimental approaches). My approach also assumes that there are no direct costs to adaptation on hosts, only a trade-off in the rates at which parasites with few or many hosts evolve (Whitlock 1996). If there are costs to adaptation, however, then my approach becomes a null model for genetic correlations across host species (i.e. advances on populations of one host species causes regression in other populations and/or other host species). Thus understanding how patterns of multiple host use and responses to host selection act in concert may help elucidate the disparity between observed and expected patterns of local adaptation in parasites.

TABLE 4.1. A revised Table 1.1 (Chapter 1), but with the methods and rationales for experimental approaches used to test for local adaptation in parasites with multiple host species (as outlined in Chapters 2 and 3). Despite fundamental differences between each approach, both should provide evidence for local adaptation, should it exist.

method	rationale	actual results		
		local adaptation	null outcome	local maladaptation
(Chapter 2) compare the success of a parasite on a primary host species, drawn from a narrow host species pool, to one drawn from a broad host species pool	local adaptation occurs when the success of the parasite on its primary local host is greater, on average, than its success on the same primary host but drawn from a broader non-local host species pool	parasites succeed better on local than non-local primary hosts of the same species	no significant differences in success of parasites on local and non-local primary hosts of the same species	parasites succeed better on non-local than local primary hosts of the same species
(Chapter 3) compare the success of a parasite on a primary host species to the same host species, but drawn from a site where they are not primary targets of selection in that locale	local adaptation occurs when the success of the parasite is greater on its local primary host species, on average, than its success on the same host species, but from a site where it is a non-target of selection in its locale	parasites succeed better on local primary host species than the same host species, but from locales where they are not primary targets of selection	no significant differences in success of parasites on local primary hosts and the same host species, but from locales where they are not primary targets of selection	parasites succeed better on non-local hosts, that are not targets of selection in their locale, than local primary hosts of the same species

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APPENDIX A

ITEM RESPONSE THEORY FOR LITERATURE SURVEYS

Effect size for a group of studies was estimated using a technique in item response theory known as the one-parameter normal ogive model for polytomous responses. Consider a collection of k studies testing local adaptation, each of which compare the outcomes of local 'sympatric' transplants (S) with non-local 'allopatric' transplants (A) of parasites and hosts (in a complete reciprocal transfer experiment, this would result in two sympatric and two allopatric comparisons). In such experiments, sample sizes are taken as the sum of all n for sympatric or allopatric tests (resulting in n^A and n^S , respectively; note these sample sizes can differ). For the i th study, I wish to observe whether the sample mean $\bar{Y}_i^A$ of the allopatric group, exceeds the sample mean $\bar{Y}_i^S$ of the sympatric group (i.e. I observe whether $\bar{Y}_i^A - \bar{Y}_i^S > 0$ for each study; Hedges & Olkin 1985). If the expected effect sizes (δ_i) for each study share a common effect (e.g., $\delta_1 = \dots = \delta_k = \delta$), my linear model implies

$$\bar{Y}_i^A - \bar{Y}_i^S \sim \mathcal{N}\left(\delta\sigma_i, \frac{\sigma_i^2}{\tilde{n}_i}\right),$$

where $\bar{Y}_i^A - \bar{Y}_i^S$ is normally distributed, with $\mathcal{N}$ denoting the normal distribution with a mean of $\delta\sigma_i$ and an error structure of $\sigma_i^2/\tilde{n}_i$ precision. Furthermore, σ_i is the common population standard deviation for the i th experimental group and

$$\tilde{n}_i = \frac{n_i^A n_i^S}{n_i^A + n_i^S}.$$

The probability of a particular study exhibiting local parasite adaptation can be expressed as function of both effect and sample size, and thus becomes

$$\text{Prob}\{\bar{Y}_i^A - \bar{Y}_i^S > 0\} = p(\delta, \tilde{n}_i) = 1 - \Phi(-\sqrt{\tilde{n}_i}\delta),$$

where $\Phi(x)$ is the standard normal cumulative distribution function, and $\sqrt{\tilde{n}_i}\delta$ is the noncentrality parameter used to estimate the sampling distribution of a particular study. Because the sample sizes are known, it should be possible to estimate δ with an outcome for each study. A research outcome, X (see below), was assigned to each study by calculating the probability of all its test(s) to exhibit local adaptation (or maladaptation), relative to the total number of tests performed. Specifically, if B number of tests were conducted in a study, then the probability of b tests showing significant evidence for a particular research outcome (either local adaptation or maladaptation) is

$$P(b) = {}_B C_b p^b q^{B-b}, \quad (1)$$

where ${}_B C_b$ is the binomial coefficient that expresses the number of ways b significant tests can be arranged within all the non-significant tests ($B - b$), p is the probability of a test yielding significant relations at the alpha level ($p = \alpha$), whereas q is the probability of a test showing non-significant relations ($1 - \alpha$). For

example, consider a study that examined eight metrics of parasite performance on sympatric and allopatric hosts (i.e. conducted eight two-tailed t -tests), but found that only one test showed significant evidence for parasites performing better on local than non-local hosts. Then the resultant probability $P(b)$, derived from the binomial expansion (equation (1)), is 0.28. Because this probability was greater than α , this test result may have resulted from random chance. Thus in this case, I summarised the study as showing no evidence for local adaptation (assigning zero to X). In other words I defined

$$X_i = \begin{cases} 1 & \text{if } P(b_i) \leq 0.05 \text{ for local adaptation} \\ 0 & \text{if } P(b_i) > 0.05 \\ -1 & \text{if } P(b_i) \leq 0.05 \text{ for local maladaptation.} \end{cases}$$

By uniting the outcomes X_i for each study into a response vector, I can estimate their gross effect size. However, because the effect cannot be solved explicitly in a formula, the effect size estimate must be expressed solely as a solution to a maximum likelihood equation. Defined as,

$$L(\delta \mid X_1, \dots, X_k) = \sum_{i=1}^k (X_i \log[p(\delta, \tilde{n}_i)] + (1 - X_i) \log[1 - p(\delta, \tilde{n}_i)]). \quad (2)$$

Because the data $X_1, \dots, X_k$ are observed and the sample sizes are known, the likelihood $L(\delta \mid X_1, \dots, X_k)$ is a function of δ alone, where it can be maximised over δ to obtain a maximum likelihood estimate ($\hat{\delta}$) (Hedges & Olkin 1985; van der Linden & Hambleton 1997). Using this method for estimating an effect has the advantage that it reaches the true value of the effect as the number of studies increases (Hedges & Olkin 1985). Specifically, $\hat{\delta}$ must be obtained numerically by

calculating the value of $L(\delta | X_1, \dots, X_k)$ for an array of possible δ values in equation (2) (but see Appendix B for machine friendly code). From this array, I selected my effect size ($\hat{\delta}$) as the estimate that gave the greatest value of likelihood. Confidence intervals were calculated using the large sample variance method derived from $\hat{\delta}$ (Hedges & Olkin 1985, p. 70).

APPENDIX B

SOURCE CODE FOR ITEM RESPONSE THEORY MODEL

The following C code is the item response theory (IRT) programme used to estimate effect sizes for groups of studies (as described by the analytical outline in Appendix A). Note, IRT is unable to estimate an effect size for a particular group if all research outcomes within this group are similar (a homogeneous outcome list; e.g., all studies within a group have null outcomes), or if groups are composed of less than four studies (van der Linden & Hambleton 1997). Moreover, if there is poor research-outcome heterogeneity within small groups (e.g., > 80% of a small group of studies have similar research outcomes), then this model will overestimate the effect size for that group; with effects greater than 0.55 or smaller than -0.55 (M. J. Lajeunesse, *personal observation*).

```
/* IRT programme for estimating the effect size for a group of studies. Input: a
text file GROUP.TXT (must be located in same folder as programme). Data in this
file should be coded as follows: each line (representing a single study) has the
experimental and control sample sizes and research outcome (e.g., 1, 0 or -1)
separated by hard tabs. Example: a GROUP.TXT file containing three studies:
```

```
122    144    1
23     22     0
1222   333    -1
```

```
Screen output of programme: the number of studies (n), and their total effect
with associated max-min 95% CI and variance (var). Coded in Borland C++ 5.4. */
```

```
#include <conio.h>
#include <fstream.h>
#include <iostream.h>
#include <iomanip.h>
#include <math.h>
```

```

double sigm(double [][][7], int);
double sigmaHelper(double, double, double, int, double[][7]);
double var(double [][][7], int, double);
double ncf(double); double ndf(double);
int allSame(double [][][7], int);

int main(void)
{
    int numberOfStudies = 0;
    const int maxNumberOfStudies = 50; // change if > 50 studies in DATA.TXT file
    double ne = 0.0, nc = 0.0, varianceOfEffect = 0.0,
           effect = 0.0, X = 0.0, studies[maxNumberOfStudies][7];

    /* File GROUP.TXT contains experimental (ne) and control (nc) sample sizes,
       and research outcome (X) for each study */
    ifstream data("GROUP.TXT");
    if (!data) {cerr << "Can't open data file..."<< endl; return 0;}

    // Clears array (studies[][]) then fills with GROUP.TXT contents
    for(int h = 0; h <= maxNumberOfStudies - 1; h++)
        for (int m = 0; m < 7; m++){studies[h][m] = 0; if (m == 3) studies[h][m]=5;}
    while (data >> ne >> nc >> X) {
        studies[numberOfStudies][0] = ne; studies[numberOfStudies][1] = nc;
        studies[numberOfStudies][2] = (ne * nc) / (ne + nc); // ñ variable
        studies[numberOfStudies][3] = X; // research outcomes {0 or 1 or -1}
        numberOfStudies++;
    }

    /* Checks if all research outcomes are similar, if so exit programme, if not
       estimate the likelihood of effect magnitude */
    if(allSame(studies, numberOfStudies) == 0){
        cerr << "Similar outcomes, cannot estimate effect!" << endl; return 0;}
    else {effect = sigm(studies, numberOfStudies);}

    // Estimates variance for effect (maximum likelihood estimate)
    varianceOfEffect = var(studies, numberOfStudies, effect);
    if (varianceOfEffect < 0) varianceOfEffect = -(varianceOfEffect);

    // Output: number of studies in GROUP.TXT and associated effects size with CI
    cout << "effect size: n = " << numberOfStudies << " "
         << effect << " 95%-CI: max " << effect + 1.96 * (sqrt(varianceOfEffect))
         << " min " << effect - 1.96 * (sqrt(varianceOfEffect))
         << " var(" << varianceOfEffect << ")" << endl;

    data.close(); return 1;
}

/* Function sigm(,): estimates effect size up to 5 decimal places through coarse
   grid trials. Assuming that the true effect size is not larger than 0.5 or
   smaller than -0.5. Returns effect size. */

double sigm(double data[][7], int size)
{
    double value = 0.0, value1 = 0.5, value2 = -0.5, deci = 0.1, preci = 0.1;

    for (int i = 0; i < 4; i++) {
        value = sigmaHelper(value1, value2, deci, size, data);
        deci = deci / 10; value1 = value + preci; value2 = value - preci;
        preci = preci / 10;
    }

    return value;
}

```

```

}
/* Function sigmaHelper(,,,): finds maximum effect size through grid comparisons
at a particular precision. Used by sigm(,). Returns coarse effect size. */

double sigmaHelper(double max, double min, double step, int size, double
info[][7])
{
    double find = 0.0, sigma = 0.0, d = -1000.0, a = 0.0, i = min;

    do {
        for(int j = 0; j <= size; j++) {
            if (info[j][3] != 5) {
                a = ncf(-(sqrt(info[j][2]) * i));
                sigma = sigma + (info[j][3] * log(1 - a) + (1 - info[j][3]) * log(a));
            }
        }
        if (sigma > d) {d = sigma; find = i;}
        a = sigma = 0; i += step;
    } while (i <= max);

    return find;
}

/* Function ncf(): Standard normal distribution function or  $\Phi()$ . Used by
sigmaHelper(,,,). Returns normal distribution value. */

double ncf(double x)
{
    double result;

    if (x < -7.0) result = ndf(x) / sqrt(1.0 + x * x);
    else if (x > 7.0) result = 1.0 - ncf(-x);
    else {
        result = 0.2316419;
        static double a[5] =
            {0.31938153, -0.356563782, 1.781477937, -1.821255978, 1.330274429};
        result = 1.0 / (1 + result * fabs(x));
        result = 1.0 - ndf(x) * (result * (a[0] + result * (a[1] + result * (a[2] +
            result * (a[3] + result * a[4])))));
        if (x <= 0.0) result = 1.0 - result;
    }

    return result;
}

/* Function ndf(): Standard normal density function. Used by ncf(). Returns
normal density value. */

double ndf(double t)
{
    return 0.398942280401433 * exp(-t * t / 2.0);
}

/* Function var(,,): Calculates the variance around the effects size (following
p. 70 of Hedges & Olkin (1985)). Returns variance of effect size. */

double var(double data[][7], int size, double sigma)
{
    const float PI = 3.14159; double final = 0.0;

    for(int i = 0; i <= size; i++) {
        if (data[i][3] != 5) {

```

```

        data[i][4] = 1-ncf(-(sqrt(data[i][2]) * sigma)); //p(effect magnitude, ñ)
        data[i][5] = sqrt(data[i][2] / (2.0 * PI)) *
            exp(-0.5 * data[i][2] * sigma * sigma);
        data[i][6] = -((data[i][2] * sigma) / (sqrt(2 * PI))) *
            exp(-0.5 * data[i][2] * sigma * sigma);
    }
}

for(int j = 0; j < size; j++) if (data[j][3] != 5)
    final = final + (data[j][5] + data[j][6] - (data[j][5] * data[j][5]) *
        ((1.0 - 2.0 * data[j][4]) / (data[j][4] * (1.0 - data[j][4]))));

final = 1.0 / final;

return final;
}

/* Function allSame(): checks if all research outcomes in a group of studies are
similar (e.g., homogeneous). Returns 0 if similar or 1 if not. */

int allSame(double list[][7], int size)
{
    int same = 0;

    for(int i = 0; i <= size; i++)
        if(list[i][3] != list[0][3])
            if (list[i][3] != 5) same = 1;

    return same;
}

```

APPENDIX C

PHENOTYPE DISCRIMINATION ANALYSIS

I wanted to determine if my results of Chapter 3.4 d) on *L. americana* attachment success were biased by the inclusion of dragonfly individuals with extreme phenotypes (defined in terms of structural size, weight, or wing cell asymmetry (FA)). Specifically in this Appendix, I wanted to make sure that a relatively homogenous sample of adults from each dragonfly species was being compared, so I could conclude species differences in mite success (if they existed), rather than apparent species differences ascribable to a few 'deviant' individuals. Of course comparisons of weight, size and wing cell FA only make sense within species.

The measurements used in this analysis were as follows. I first counted the number of wing cells between the nodus and pterostigma for both the left and right forewings (to obtain a measure of wing cell asymmetry). I included FA because it can act as a measure of developmental instability and perhaps condition before adulthood (Bonn *et al.* 1996; Leung & Forbes 1997). I also measured the length of the right forewing from the nodus to wing tip using digital callipers (accurate to the nearest 0.01 mm; Miyamoto digital callipers) and recorded weight (Mettler AE100 Digital Scale).

To find individuals with extreme phenotypes, I used a Mahalanobis outlier analysis. This analysis is a useful way of determining the similarity of dragonfly individuals that share a group of common characteristics (e.g., weight, wing-length and FA), where outliers represent individuals that do not fit (or contribute) to multivariate descriptions relating to the groups tested (in my case descriptions within species). Each individual dragonfly is designated a Mahalanobis distance unit (MDU), which is measured in terms of standard deviations from the multivariate mean. Following Duntelman (1984), individuals were considered as potential outliers if they deviated more than three MDU's; any measure above three would suggest a < 1% probability of belonging (or contributing) to that group. All analyses were performed in Jmp 4.0 (2000 S.A.S. Institute Inc.).

The Mahalanobis analysis revealed three potential phenotypic outliers: specifically, one *N. bella* male (3.73 MDU) and two *L. frigida* males (3.28 and 3.17 MDU). Rerunning my analyses of Chapter 3.4 d) after excluding these outliers, however, did not change my initial findings. Briefly, the difference in mite mortality between host species was retained (non-parametric ANCOVA: $\chi^2 = 16.23$, d.f. = 1, $p < 0.001$; with covariate: number of attached mites: $\chi^2 = 3.79$, d.f. = 1, $p = 0.058$). Likewise, there were no differences in the number of mites attaching to each dragonfly species (non-parametric ANCOVA: $\chi^2 = 1.36$, d.f. = 1, $p = 0.246$; with covariates: timing of trial, $\chi^2 = 8.17$, d.f. = 1, $p = 0.004$ and number of mites challenged, $\chi^2 = 3.75$, d.f. = 1, $p = 0.053$). Thus, the inclusion of phenotypic outliers did not bias my initial results that *L. frigida* appeared to be a more suitable host species than *N. bella* dragonflies.