Molecular Ecology (2005) **14**, 3317–3324 doi: 10.1111/j.1365-294X.2005.02684.x

Mitochondrial DNA analysis of the bark beetle predator *Thanasimus dubius* F. (Coleoptera: Cleridae) reveals regional genetic differentiation

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Abstract

The checkered beetle, *Thanasimus dubius* F., is an important predator of scolytid bark beetles that attack conifers. Relatively few studies exist that have addressed the population genetics of predatory beetles, especially those with potential as biological control agents. This study was conducted to investigate the population genetics of *T. dubius* across a large part of its range in the eastern United States. A 464-base pair portion of the mitochondrial cytochrome *c* oxidase subunit I was sequenced for 85 individuals resulting in 60 haplotypes. Analysis of molecular variance was conducted on the resulting haplotypes for all populations and as a hierarchical analysis between populations defined as broad-scale northern and southern groups. Results indicate a significant overall $\Phi_{ST} = 0.220$ ($P < 0.001$) for all populations with the hierarchical analysis revealing that this significant Φ_{ST} is due to structuring of the populations between northern and southern regions ($\Phi_{CT} = 0.388$, $P < 0.009$). The observed genetic structure is possibly due to the discontinuous distribution of pine trees, which act as hosts for the prey of *T. dubius*, which has occurred historically in the central region of the United States that has been covered by prairie.

Keywords: Cleridae, Coleoptera, mtDNA cytochrome oxidase I, population genetics, *Thanasimus dubius*

Received 11 April 2005; revision accepted 22 June 2005

Introduction

The checkered beetle *Thanasimus dubius* F. (Coleoptera: Cleridae) has a large geographical range, extending from Texas (Thatcher & Pickard 1966) and the states adjoining the Gulf of Mexico north to the Great Lakes and into Canada (Schenk & Benjamin 1969). Across its range, *T. dubius* is an important predator of scolytid bark beetles that attack conifers, especially the southern pine beetle, *Dendroctonus frontalis* Zimm. (Coleoptera: Scolytidae) (Thatcher & Pickard 1966; Reeve 1997; Reeve & Turchin 2002). This predator also attacks several species of *Ips* (Coleoptera: Scolytidae) including *Ips avulsus*, *Ips calligraphus*, *Ips grandicollis*, and *Ips pini* (Mignot & Anderson 1969; Aukema & Raffa 2002). *T. dubius* has also been reported from spruce stands in Great Lakes sites, Utah, and Alaska, where it is apparently associated with the spruce beetle *Dendroctonus rufipennis* (Kirby) (Gara *et al*. 1995; Bentz & Munson 2000; Haberkern & Raffa 2003). Several of these prey species are destructive forest pests that cause significant economic losses, especially *D. frontalis* and *D. rufipennis*.

The interactions between *T. dubius* and its bark beetle prey have been well documented. The prey species in this system uses various aggregation pheromones to attract mates and apparently coordinate their attacks on host trees (Payne 1980; Borden 1982). Adult *T. dubius* are also attracted by these chemicals (Vité & Williamson 1970; Herms *et al*. 1991; Raffa 2001), arriving almost simultaneously on the host tree, where they feed upon adult bark beetles and oviposit in bark crevices (Thatcher & Pickard 1966; Dixon & Payne 1979). The larvae of *T. dubius* are also predaceous and feed on immature bark beetles within the host tree (Thatcher & Pickard 1966; Aukema & Raffa 2002).

Because *T. dubius* is widely distributed, it interacts with different prey species, and hence pheromones, in different

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portions of its range. Therefore, regional differences in *T. dubius* response to prey pheromones likely exist. For example, the southern pine beetle *D. frontalis* can be extremely abundant in the southern United States, and in this portion of its range *T. dubius* responds strongly to *D. frontalis* pheromones, while the responses to *Ips* pheromones are weak or absent (Billings & Cameron 1984; Billings 1985). In more northern locations where *D. frontalis* is absent, strong responses to *Ips* pheromones have been observed (Raffa & Klepzig 1989; Herms *et al*. 1991; Erbiligin & Raffa 2000). A geographical survey of the relative response of *T. dubius* to these prey pheromones further suggests the response to *Ips* pheromones is negligible at locations where *D. frontalis* is abundant (J. D. Reeve *et al*., unpublished data). The exact nature of these geographical patterns is unknown, but given the distances involved, it is possible they involve genetic differences in pheromone response among *T. dubius* populations.

The objectives of this study were to determine the levels of genetic differentiation and estimate the amount of gene flow occurring among populations of *T. dubius* using sequence data from a mitochondrial gene. Because *T. dubius* shows geographical variation in its response to prey pheromones, this study can be used to determine if differential response to pheromones may be due to genetic differences among locations, which to our knowledge, has not been addressed in the literature. We also note there are relatively few studies of genetic structure for biological control agents, especially insect predators, despite their ecological and economic importance. This is in contrast to the vast body of literature for phytophagous insects, where significant genetic differences among populations from different locations are routinely found (see Mopper & Strauss 1998 for a review). Results of this analysis give the first information regarding genetic structure of populations of *T. dubius* across its geographical range and serve as a foundation for studies of regional behavioural and ecological differences within this species.

Materials and methods

Insect collection

Adult *Thanasimus dubius* individuals (n = 85) from distinct geographical locations (Fig. 1) representing 10 states were sampled from November 2001 to July 2003 (Table 1). The predators were collected using multiple funnel traps (Lindgren 1983) baited with the aggregation pheromone

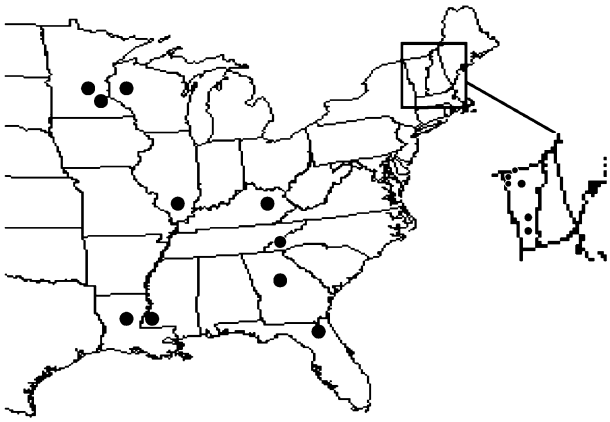

Fig. 1 Map of the eastern United States with locations from which *Thanasimus dubius* were sampled indicated with a black circle (see Table 1 for descriptions).

Table 1 Sampling locations, number of individuals screened (N), and date of collection for *Thanasimus dubius* screened at a 464-bp fragment of the mitochondrial COI gene are provided

Population group	State	County	*N*	Date
North (SPB absent)	Minnesota	Dakota Co.	5	July 2003
		Hennepin Co.	4	
	Wisconsin	Dunn Co.	4	August 2002
	Vermont	Washington Co.	3	May–July 2003
		Grand Isle Co.	1	
		Orange Co.	5	
		Chittenden Co.	1	
		Franklin Co.	1	
		Windsor Co.	2	May–June 2002
South (SPB present)	Illinois	Johnson Co.	10	June–July 2002
	Kentucky	Pulaski Co.	9	June 2002
	North Carolina	Cherokee Co.	9	June 2002
	Louisiana	Rapides Pa.	9	November 2001
	Mississippi	Amite Co.	10	July 2002
	Georgia	Putnam Co.	8	June 2002
	Florida	Alachua Co.	4	June–August 2002

of *Dendroctonus frontalis*, frontalin (PheroTech, Inc.), and turpentine made from southern pine species, a combination highly attractive to *T. dubius* (Vité & Williamson 1970; Billings 1985). The traps were placed within pine–deciduous forests containing pine species favoured by the prey species. Adult *T. dubius* were also collected by hand from trees undergoing attack by *D. frontalis*. Upon collection, specimens were kept alive until they reached the laboratory where they were stored at −80 °C or were placed immediately in > 70% ethanol.

Polymerase chain reaction and DNA sequencing

Genomic DNA was extracted from legs and/or slices of partial abdomen to avoid possible contamination by recent prey items. DNA was extracted using a high-salt DNA extraction protocol modified from Pogson *et al*. (1995) by Anderson (2000). Extracted DNA pellets were stored at –20 °C.

The mitochondrial cytochrome *c* oxidase subunit I gene (COI) was PCR amplified (polymerase chain reaction) and sequenced using universal primers (HCOI/LCOI) from Folmer *et al*. (1994). PCR amplifications were carried out in 25-μL volumes containing 1× HotStar*Taq* Master Mix (QIAGEN), 2.5 μL of 10 μM primer mix, approximately 1–10 ng DNA template, and water. Reactions were run on a Stratagene RoboCycler with the following profile: 95 °C for 15 min; 35 cycles of 94 °C for 1 min, 40–45 °C for 1 min, 72 °C for 1 min 30 s; 72 °C for 7 min. PCR products were stored at –20 °C until further analysis. PCR products were gel-purified using 1% agarose gels and a QIAquick Gel Extraction Kit (QIAGEN).

Gel-purified PCR products were sequenced in both directions using BigDye Terminator mix version 3.0 (PE Applied Biosystems) following a modified protocol for 1/2 and 1/8 reactions. Sequencing reactions were carried out either in 10-μL volumes containing 1 μL of 3.2 μM primer, 4 μL of BigDye Terminator mix, and a template/sterile water mixture containing 5–20 ng template or 20-μL volumes containing 1 μL of 3.2 μM primer, 1 μL of BigDye Terminator mix, 3.5 μL of 5× sequencing buffer, and a template/sterile water mixture containing 5–20 ng template.

Cycle-sequencing reactions were run on a GeneAmp PCR System 9700 thermal cycler (PE Applied Biosystems) following the manufacturer's protocol except that the number of cycles was increased to 99. Purification of the sequencing reactions was accomplished using Centri-Sep spin columns (Princeton Separations) packed with Sephadex G-50 Fine resin (Amersham Biosciences).

Purified cycle-sequencing reactions were electrophoresed on an ABI PRISM 377 DNA sequencer (Applied Biosystems). Sequences of each haplotype present are available in GenBank under Accession nos AY790473–AY790532.

Data analysis

DNA sequences generated in both directions (forward and reverse primer sequences) were assembled and edited to obtain a consensus sequence for each sample using SEQUENCHER version 4.1.2 (GeneCode, Inc.); consensus sequences were aligned using CLUSTAL_X version 1.8 (Thompson *et al*. 1997) and SE-AL version 1.0a1 (Rambaut 1995). Unique haplotypes were defined using the program COLLAPSE version 1.1 (D. Posada, available at http://darwin.uvigo.es/) and verified by MEGA2 (Kumar *et al*. 2001). MEGA version 2.0 was also used to calculate the number of variable sites and nucleotide composition. A haplotype network was constructed using the 95% parsimony criterion as implemented by the program TCS (Clement *et al*. 2000). The absolute number of migrants ($M = Nm$) exchanged between populations was estimated in ARLEQUIN version 2.000 (Schneider *et al*. 2000) using methods outlined in Slatkin (1991).

Individuals sampled from one location were combined together to form the initial groups for comparisons. Individuals were sampled from multiple proximate locations in Minnesota and Vermont. These individuals were pooled to form a single sample if no significant differentiation was observed among them in initial analysis (data not reported). Thus, 10 groups were constructed initially. The 10 groups are Minnesota, Wisconsin, Vermont, Illinois, Kentucky, North Carolina, Louisiana, Mississippi, Georgia, and Florida; these groups represent samples from distinct forests. Nucleotide sequence diversity and haplotype diversity were calculated within each geographical sample with ARLEQUIN version 2.000.

Estimates of genetic differentiation were calculated following the AMOVA method of Excoffier *et al*. (1992) as implemented by ARLEQUIN version 2.000. Pairwise distances were calculated among all haplotypes using the Tajima and Nei model as calculated by ARLEQUIN. Analysis of molecular variance was performed on these pairwise distances for two hierarchical groupings of the data. The first level of the AMOVA compared individuals within defined geographical samples. The second level consisted of comparisons among primary geographical samples, which consisted of individuals combined from all geographically distinct sample locations. Estimates of Φ_{ST}, an analogue of F_{ST}, were calculated overall for all samples and pairwise between all possible geographical pairs. Statistical significance values were calculated by permutations of haplotypes among geographical samples, with sequential Bonferroni correction of $\alpha = 0.05$ performed for the pairwise comparisons.

The final hierarchical grouping combined the initial geographical samples into more broad-scale units. These units were defined by grouping samples based on the presence or absence of the southern pine beetle. Specifically, samples

from Minnesota, Wisconsin, and Vermont were combined to form a northern group, a region which lacks southern pine beetle; samples from Illinois, Kentucky, North Carolina, Louisiana, Mississippi, Georgia, and Florida were combined to form a southern group, a region which has southern pine beetle. Estimates of Φ_{CT} were calculated between the two broad-scale groups. Statistical significance values were calculated by permutation of haplotypes among broad-scale groups with statistical significance set at $\alpha = 0.05$.

Results

Sequence variation

Sequences of a 464-bp fragment of the mtDNA COI gene were obtained for 85 individuals sampled from 10 states. Merging identical sequences resulted in 60 unique haplotypes. Of the 464 sites, 56 were variable. The amplified segment of COI was AT rich (A: 35.9%, C: 15.7%, G: 17.1%, T: 31.3%). Though at least 10 individuals were collected from Wisconsin and Florida, final sample sizes were less than 10 because initial PCRs did not work consistently, likely due to tissue preservation conditions.

Data analysis

The relative haplotype frequencies for each population were low with the exception of the northern populations of Minnesota, Wisconsin, and Vermont (Table 2). These three populations share haplotype 4 at a high frequency. Haplotype 3 was shared among the southern populations of Illinois, Mississippi, Georgia, and Florida. There were several rare haplotypes that each occurred in only one population. Nucleotide sequence diversity (Table 3) was similar for all samples (range 0.010–0.014) except the Minnesota and Wisconsin samples, which had lower

Table 2 Relative haplotype frequencies among populations sampled with the total number of individuals from each location screened in parentheses. For each haplotype, a unique label is provided in bold and the frequency of the haplotype follows

Minnesota (9)	Wisconsin (4)	Vermont (13)	Illinois (10)	Kentucky (9)	North Carolina (9)	Louisiana (9)	Mississippi (10)	Georgia (8)	Florida (4)
—	—	—	**3** 0.2	—	—	—	**3** 0.1	**3** 0.25	**3** 0.25
4 0.667	**4** 0.75	**4** 0.462	—	—	—	—	—	—	—
—	**10** 0.25	**10** 0.154	—	—	**10** 0.111	—	—	—	—
—	—	—	—	—	**21** 0.111	**21** 0.111	**21** 0.1	—	—
52 0.111	—	**26** 0.0769	**18** 0.2	**13** 0.111	**1** 0.111	**35** 0.111	**6** 0.1	**2** 0.125	**5** 0.25
53 0.111	—	**55** 0.0769	**19** 0.1	**22** 0.111	**14** 0.111	**36** 0.111	**7** 0.1	**15** 0.125	**12** 0.25
54 0.111	—	**56** 0.0769	**34** 0.1	**24** 0.111	**17** 0.111	**37** 0.111	**8** 0.1	**16** 0.125	**59** 0.25
—	—	**57** 0.0769	**43** 0.1	**28** 0.111	**23** 0.111	**38** 0.111	**9** 0.1	**20** 0.125	—
—	—	**58** 0.0769	**44** 0.1	**30** 0.111	**25** 0.111	**39** 0.111	**11** 0.1	**29** 0.125	—
—	—	—	**45** 0.1	**47** 0.111	**31** 0.111	**40** 0.111	**27** 0.1	**33** 0.125	—
—	—	—	**46** 0.1	**48** 0.111	**32** 0.111	**41** 0.111	**50** 0.1	—	—
—	—	—	—	**49** 0.111	—	**42** 0.111	**51** 0.1	—	—
—	—	—	—	**60** 0.111	—	—	—	—	—

Table 3 The number of *Thanasimus dubius* screened from each geographical location is provided with nucleotide diversity, number of haplotypes observed, and haplotype diversity. Standard deviations are provided for nucleotide diversity and haplotype diversity

State	No. of individuals	Nucleotide diversity ± SD	No. of haplotypes	Haplotype diversity ± SD
Northern Group				
Minnesota	9	0.005 ± 0.003	4	0.583 ± 0.183
Wisconsin	4	0.001 ± 0.001	2	0.500 ± 0.265
Vermont	13	0.010 ± 0.006	7	0.795 ± 0.109
Southern Group				
Illinois	10	0.013 ± 0.007	8	0.956 ± 0.059
Kentucky	9	0.014 ± 0.008	9	1.000 ± 0.052
North Carolina	9	0.013 ± 0.008	9	1.000 ± 0.052
Louisiana	9	0.013 ± 0.008	9	1.000 ± 0.052
Mississippi	10	0.012 ± 0.007	10	1.000 ± 0.045
Georgia	8	0.014 ± 0.008	7	0.964 ± 0.077
Florida	4	0.013 ± 0.009	4	1.000 ± 0.177

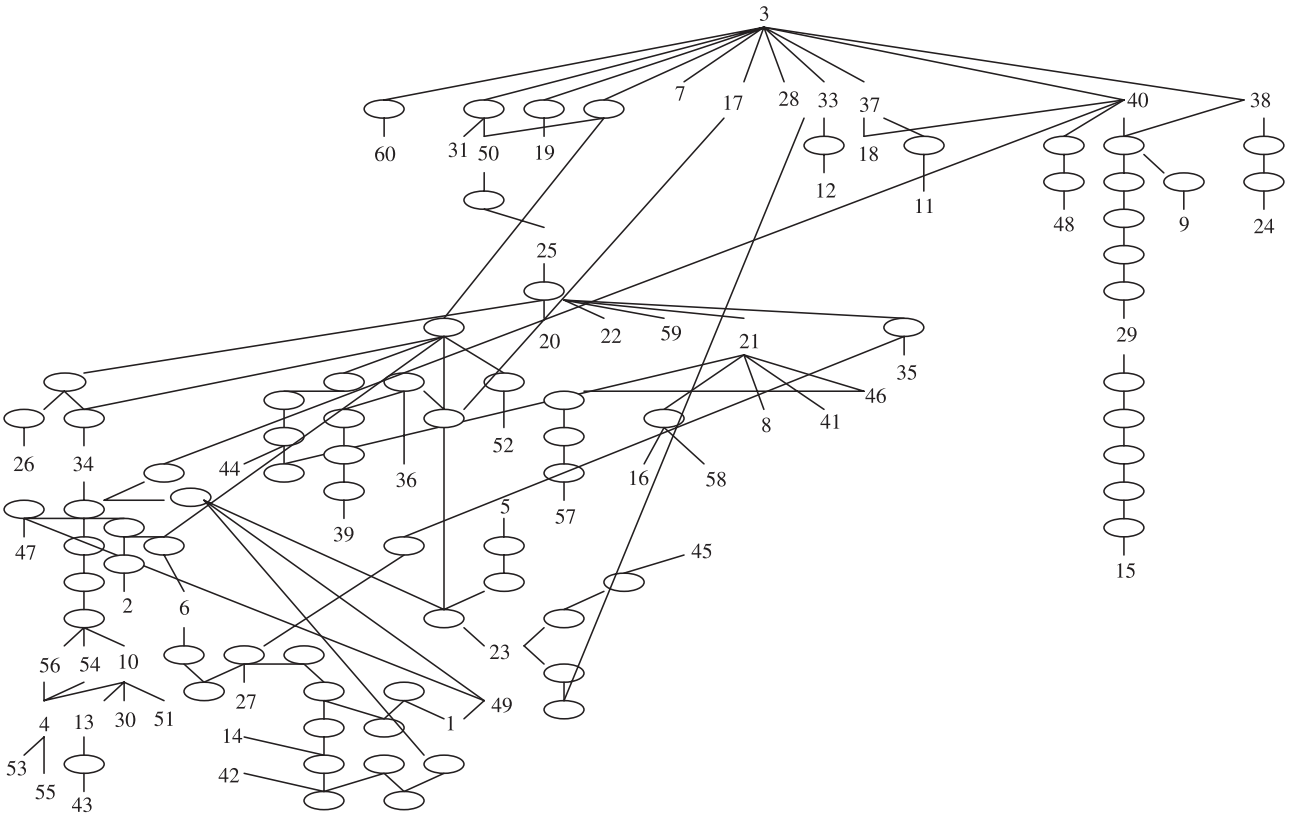

Fig. 2 A 95% parsimony network of 60 haplotypes obtained by sequencing a 464-bp fragment of the mtDNA COI gene in 85 individuals of *Thanasimus dubius* as generated by TCS (Clement *et al*. 2000). Empty circles represent missing (or unsampled) haplotypes. Numbers are provided indicating observed haplotypes. Each segment connecting haplotypes represents a single mutation event.

Table 4 Estimates of genetic differentiation calculated by AMOVA employing Tajima–Nei distances among haplotypes. Analysis conducted among populations as one hierarchical group (One group) that includes all states sampled and with an additional hierarchical group (Two groups) corresponding to broad-scale northern and southern geographical areas. Areas outside the range of the southern pine beetle characterize the northern group, while the southern group is characterized by presence of the southern pine beetle. In both, the amount of variation occurring among categories and the estimate of genetic differentiation is provided. An asterisk denotes statistical significance at $\alpha = 0.05$

Hierarchy	Categories	% variation	Φ estimate
One group	Among populations	21.98	$\Phi_{ST} = 0.220$*
	Within populations	78.02	
Two groups	Among groups	38.80	$\Phi_{CT} = 0.388$*
	Among populations within groups	−0.98	$\Phi_{ST} = 0.378$*
	Within populations	62.18	

diversity observed (0.005 and 0.001). Haplotype diversity (Table 3) was also similar for all samples (range 0.795–1) except Minnesota and Wisconsin (0.583 and 0.5). The samples that formed the northern group in hierarchical analysis were characterized by having lower nucleotide diversity and lower haplotype diversity when compared to the southern group.

TCS generated a 95% parsimony haplotype network for the 60 haplotypes (Fig. 2). The network contains many loops, which are indicative of homoplasies and the presence of reverse/parallel mutations (Posada & Crandall 2001). There were no abundant haplotypes, and the highest outgroup weight detected was 0.10 (haplotype 3).

AMOVA detected a significant overall Φ_{ST} (0.220, $P < 0.001$) when comparing genetic variation among populations (Table 4). The great majority of the variation was within rather than among populations (within 78.02%, among 21.98%). Hierarchical AMOVA conducted between the northern

Table 5 Pairwise estimates of Φ_{ST} for each population pair are provided below the diagonal with an asterisk denoting statistical significance after sequential Bonferroni correction of $\alpha = 0.05$. Pairwise estimates of gene flow (*Nm*), as calculated by ARLEQUIN are provided above the diagonal

	Minnesota	Wisconsin	Vermont	Illinois	Kentucky	North Carolina	Louisiana	Mississippi	Georgia	Florida
Minnesota	***	Inf.	Inf.	0.53	0.83	0.69	0.53	0.58	0.43	0.34
Wisconsin	−0.08	***	Inf.	0.51	0.85	0.64	0.49	0.54	0.42	0.30
Vermont	−0.02	−0.05	***	0.95	1.54	1.29	0.96	1.03	0.75	0.74
Illinois	0.48*	0.49	0.34*	***	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.
Kentucky	0.38*	0.37	0.25	−0.03	***	Inf.	35.79	Inf.	12.63	Inf.
North Carolina	0.42	0.44	0.28	−0.01	−0.03	***	Inf.	Inf.	14.31	Inf.
Louisiana	0.48*	0.51	0.34	−0.03	0.01	−0.06	***	Inf.	132.3	Inf.
Mississippi	0.46*	0.48	0.33*	−0.04	−0.02	−0.03	−0.05	***	Inf.	Inf.
Georgia	0.54*	0.54	0.40	−0.01	0.04	0.03	0.004	−0.001	***	Inf.
Florida	0.59	0.63	0.40	−0.04	−0.02	−0.01	−0.04	−0.03	−0.05	***

and southern groups detected a significant Φ_{CT} (0.388, $P < 0.009$) between the northern and southern populations (Table 4). The comparison among groups (Φ_{CT}) accounted for all the variation detected among populations. Seven pairwise comparisons of Φ_{ST} were statistically significant after sequential Bonferroni correction for multiple tests at $\alpha = 0.05$ (Table 5). Significant pairwise comparisons are only observed in comparisons between samples from the northern group and the southern group. No significant comparisons were observed within broad-scale groups. However, not all samples from the southern group were significantly differentiated from samples in the northern group.

Estimates of *M* for all population pairs revealed a greater magnitude in exchange of migrants within each broad-scale geographical group than between geographical groups (Table 5). This implies that within each geographical region, there is substantial gene flow; however, low values of *M* between groups imply that little gene flow is occurring between regions.

Discussion

This genetic investigation of *Thanasimus dubius* revealed significant levels of genetic differentiation among populations. The fragment of COI was highly variable with 60 haplotypes being observed in the 85 individuals sampled. A statistically significant estimate of Φ_{ST} and few shared haplotypes were observed among 10 populations, with a hierarchical estimate of genetic differentiation calculated among two groups, north and south, accounting for the genetic differentiation detected. The significant differentiation detected among groups and the lack of significant differentiation within groups suggest that *T. dubius* may be characterized by two geographically distinct populations, roughly corresponding to northern vs. southern regions, with a low level of gene flow occurring between them. The lack of detectable differentiation within each region and population suggests that higher amounts of gene flow are occurring at these levels.

The placement of Illinois, Kentucky, and North Carolina in the southern group was based on the presence of the southern pine beetle in these locations. These sample regions did not share the haplotype shared by the other members of the southern region. It may be that there is additional fine-scale structuring of *T. dubius* within the categories defined by presence/absence of the southern pine beetle. However, classifying groups based on this factor did account for the variation observed among samples.

The 95% parsimony network did not distinguish distinct northern and southern groupings of haplotypes. Some northern haplotypes (4, 10, 53, 54, 55, 56) are grouped together, but they are also clustered with southern haplotypes (13, 30, 43, 51). All other northern haplotypes are scattered throughout the network with little structure apparent. This lack of distinct clustering is likely due to incomplete separation of haplotypes among the northern and southern groups caused by migration among groups or recent separation of the groups.

The genetic differentiation observed between the northern and southern regions may be due to discontinuous pine tree habitat between regions. *T. dubius* is ultimately limited by the habitat requirements of its prey species, which utilize various pine tree species as hosts, including shortleaf (*Pinus echinata*), jack (*Pinus banksiana*), slash (*Pinus elliottii*), longleaf (*Pinus palustris*), red (*Pinus resinosa*), pitch (*Pinus rigida*), eastern white (*Pinus strobus*), and loblolly (*Pinus taeda*) pine. Examination of the current distributions of these pine species indicates a large gap in their distribution in central Illinois, Indiana, and Ohio (Burns & Honkala 1990; Prentice *et al*. 1991). This gap may act as a partial barrier to gene flow between the northern and southern populations of *T. dubius*.

The lack of population structure within northern and southern regions is not surprising given the dispersal

capabilities of *T. dubius*. Cronin *et al*. (2000) performed a mark–recapture study to investigate the movements of *T. dubius* and found a median dispersal distance of about 1.25 km, with some individuals moving considerably farther. In phytophagous insects, dispersal of this magnitude was correlated with high levels of gene flow (Peterson & Denno 1998), and this appears to be the case with *T. dubius* as well.

The genetic differentiation found between the northern and southern regions suggests that differences in pheromone response observed in these locations could be genetic in origin, especially responses to *Ips* pheromones. However, within each region, we would expect gene flow to homogenize pheromone responses. Studies are currently underway to determine if regional differences in pheromone response are genetic or behavioural in origin.

This study differs from previous population genetics studies of predatory beetles, finding significant genetic differentiation in *T. dubius*. Three studies using allozyme electrophoresis have found high levels of gene flow among populations of highly mobile coccinellid species whose ranges cover most of the continental United States. A high level of gene flow was found among North American populations of introduced *Coccinella septempunctata* (Krafsur *et al*. 1992). High levels of gene flow were detected among populations from Georgia, Virginia, Delaware, Rhode Island, Arkansas, Illinois, Iowa, and Oregon for another introduced exotic, *Harmonia axyridis* (Krafsur *et al*. 1997). Also, no significant differentiation was detected among populations of *Coleomegilla maculata* sampled from Delaware and Iowa (Krafsur *et al*. 1995). These studies attribute the lack of genetic differentiation to the aggregation behaviour exhibited by these beetles during dormancy or diapause (Hagen 1962). Mating oftentimes occurs in these aggregations before dispersal (Hagen 1962), which could act to homogenize separate populations. This type of aggregation behaviour is not exhibited by *T. dubius*.

The pattern of genetic differentiation found among northern and southern populations of *T. dubius* differs from that found in one of its prey species, *Ips pini*. Cognato *et al*. (1999) investigated gene flow in *Ips pini* by sequencing a 354-bp fragment of the mitochondrial COI gene for 217 individuals from 22 North American populations. The study found moderate gene flow among all populations and regions. In the east region (New York, Rhode Island, Maryland, Wisconsin, and Minnesota), where both *I. pini* and *T. dubius* are found, a moderate to high level of gene flow was observed and no genetic structure for *I. pini* was inferred.

As previously mentioned, investigations into the use of the predator *T. dubius* as a biological control agent are currently underway. An artificial diet has been developed for *T. dubius* (Reeve *et al*. 2003), and it may eventually be feasible to augment field population using mass-reared individuals. However, the presence of regional genetic structure in *T. dubius* should be considered before individuals are transplanted among regions. An attempt should be made to keep individuals used for biological control within the broad-scale geographical region from which they originated, because they likely will possess adaptations to the prey pheromones commonly encountered. The lack of genetic differentiation among states within regions suggests that individuals from one state within a region could be transferred to another location within the same region. It is possible that additional information concerning the use of *T. dubius* as a biological control agent could be obtained by supplementing the findings of this study with other genetic markers such as microsatellites.

Acknowledgements

We thank M. Burnham, S. Smith, B. Ayres, S. Martinson, M. Ayres, B. Strom, J. Foltz, and D.T. Almquist for providing samples. D. Crockett and J. Cramer from the Daniel Boone National Forest, D. Stiles from the Nantahala National Forest, and E. Caldwell and M. Aparicio from the Oconee National Forest provided assistance in finding locations to trap *T. dubius*. E. Heist provided use of the ABI 377. N. Smith provided invaluable field assistance. A. Schrey provided help with data analyses and reviewed an early version of the manuscript. We acknowledge three anonymous reviewers who provided valuable comments on a previous version of this manuscript. This research was supported in part by a cooperative agreement with the Southern Research Station, SRS-4501, USDA Forest Service and a Competitive Research Grant from the SIUC University Women's Professional Advancement and Women's Studies.

References

Anderson FE (2000) Phylogeny and historical biogeography of the loliginid squids (Mollusca: Cephalopoda) based on mitochondrial DNA sequence data. *Molecular Phylogenetics and Evolution*, **15**, 191–214.

Aukema BH, Raffa KF (2002) Relative effects of exophytic predation, endophytic predation, and intraspecific competition on a subcortical herbivore: consequences to the reproduction of *Ips pini* and *Thanasimus dubius*. *Oecologia*, **133**, 483–491.

Bentz BJ, Munson AS (2000) Spruce beetle population suppression in northern Utah. *Western Journal of Applied Forestry*, **15**, 122–128.

Billings RF (1985) Southern pine bark beetles and associated insects: effects of rapidly-released host volatiles on response to aggregation pheromones. *Zeitschrift für Angewandt Entomologie*, **99**, 483–491.

Billings RF, Cameron RS (1984) Kairomonal responses of Coleoptera, *Monochamus titillator* (Cerambycidae), *Thanasimus dubius* (Cleridae), and *Temnochila virescens* (Trogositidae), to behavioral chemicals of southern pine bark beetles (Coleoptera: Scolytidae). *Environmental Entomology*, **13**, 1542–1548.

Borden JH (1982) Aggregation pheromones. In: *Bark Beetles in North American Conifers: A System for the Study of Evolutionary Biology* (eds Mitton JB, Sturgeon KB), pp. 74–139. University of Texas Press, Austin, Texas.

Burns RM, Honkala BH (1990) *Silvics of North America*, vol. 1: *Conifers*. USDA Forest Service Agriculture Handbook 654. Washington, D.C.

Clement M, Posada D, Crandall KA (2000) TCS: a computer program to estimate gene genealogies. *Molecular Ecology*, **9**, 1657–1659.

Cognato AI, Seybold SJ, Sperling FAH (1999) Incomplete barriers to mitochondrial gene flow between pheromone races of the North American pine engraver, *Ips pini* (Say) (Coleoptera: Scolytidae). *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **266**, 1843–1850.

Cronin JT, Reeve JD, Wilkens R, Turchin P (2000) The pattern and range of movement of a checkered beetle predator relative to its bark beetle prey. *Oikos*, **90**, 127–138.

Dixon WN, Payne TL (1979) *Sequence of arrival and spatial distribution of entomophagous and associate insects in southern pine beetle-infested trees*. Texas Agricultural Experiment Station MP-1432, College Station, Texas.

Erbiligin N, Raffa KF (2000) Opposing effects of host monoterpenes on responses by two sympatric species of bark beetles to their aggregation pheromones. *Journal of Chemical Ecology*, **26**, 2527–2548.

Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics*, **131**, 479–491.

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, **3**, 294–299.

Gara RI, Werner RA, Whitmore MC, Holsten EH (1995) Arthropod associates of the spruce beetle *Dendroctonus rufipennis* (Kirby) (Col., Scolytidae) in spruce stands of south-central and interior Alaska. *Journal of Applied Entomology*, **119**, 585–590.

Haberkern KE, Raffa KF (2003) Phloeophagous and predaceous insects responding to synthetic pheromones of bark beetles inhabiting white spruce stands in the Great Lakes region. *Journal of Chemical Ecology*, **29**, 1651–1663.

Hagen KS (1962) Biology and ecology of predaceous coccinellidae. *Annual Review of Entomology*, **7**, 289–326.

Herms DA, Haack RA, Ayres BD (1991) Variation in semiochemical-mediated prey–predator interaction: *Ips pini* (Scolytidae) and *Thanasimus dubius* (Cleridae). *Journal of Chemical Ecology*, **17**, 1705–1714.

Krafsur ES, Obrycki JJ, Flanders RV (1992) Gene flow in populations of the seven-spotted lady beetle, *Coccinella septempunctata*. *Journal of Heredity*, **83**, 440–444.

Krafsur ES, Obrycki JJ, Schaefer PW (1995) Genetic heterozygosity and gene flow in *Coleomegilla maculata* De Geer (Coleoptera: Coccinellidae). *Biological Control*, **5**, 104–111.

Krafsur ES, Kring TJ, Miller JC *et al.* (1997) Gene flow in the exotic colonizing ladybeetle *Harmonia axyridis* in North America. *Biological Control*, **8**, 207–214.

Kumar S, Tamura K, Jakobsen IB, Nei M (2001) MEGA2: molecular evolutionary genetics analysis software. *Bioinformatics*, **17**, 1244–1245.

Lindgren BS (1983) A multiple funnel trap for scolytid beetles (Coleoptera). *Canadian Entomologist*, **115**, 299–302.

Mignot EC, Anderson RF (1969) Bionomics of the bark beetle predator, *Thanasimus dubius* Fab. (Coleoptera: Cleridae). *Entomological News*, **80**, 305–310.

Mopper S, Strauss SY (1998) *Genetic Structure and Local Adaptation in Natural Insect Populations: Effects of Ecology, Life History, and Behavior*. Chapman & Hall, New York.

Payne TL (1980) Life history and habits. In: *The Southern Pine Beetle* (eds Thatcher RC, Searcy JL, Coster JE, Hertel GD), pp. 7–28. USDA Forest Service Technical Bulletin 1631.

Peterson MA, Denno RF (1998) Life-history strategies and the genetic structure of phytophagous insect populations. In: *Genetic Structure and Local Adaptation in Natural Insect Populations: Effects of Ecology, Life History, and Behavior* (eds Mopper S, Strauss SY), pp. 263–322. Chapman & Hall, New York.

Pogson GH, Mesa KA, Boutilier RG (1995) Genetic population structure and gene flow in the Atlantic cod *Gadus morhua*: a comparison of allozymes and nuclear RFLP loci. *Genetics*, **139**, 375–385.

Posada D, Crandall KA (2001) Intraspecific gene genealogies: trees grafting into networks. *Trends in Ecology & Evolution*, **16**, 37–44.

Prentice IC, Bartlein PJ, Webb T III (1991) Vegetation and climate change in eastern North America since the last glacial maximum. *Ecology*, **72**, 2038–2056.

Raffa KF (2001) Mixed messages across multiple trophic levels: the ecology of bark beetle chemical communication systems. *Chemoecology*, **11**, 49–65.

Raffa KF, Klepzig KD (1989) Chiral escape of bark beetles from predators responding to a bark beetle pheromone. *Oecologia*, **80**, 566–569.

Rambaut A (1995) *SE-AL: Sequence Alignment Program, version v1.d1*. Available at http://evolve.zoo.ox.ac.uk/

Reeve JD (1997) Predation and bark beetle dynamics. *Oecologia*, **112**, 48–54.

Reeve JD, Rojas MG, Morales-Ramos JA (2003) Artificial diet and rearing methods for *Thanasimus dubius* (Coleoptera: Cleridae), a predator of bark beetles (Coleoptera: Scolytidae). *Biological Control*, **27**, 315–322.

Reeve JD, Turchin P (2002) Evidence for predator–prey cycles in a bark beetle. In: *Population Cycles: Evidence for Trophic Interactions* (ed. Berryman AA), pp. 92–108. Oxford University Press, New York.

Schenk JA, Benjamin DM (1969) Notes on the biology of *Ips pini* in central Wisconsin jack pine forests. *Annals of the Entomological Society of America*, **62**, 480–485.

Schneider S, Roessli D, Excoffier L (2000) *ARLEQUIN, version 2.000: a software for population genetics data analysis*. Genetics and Biometry Laboratory, Department of Anthropology, University of Geneva, Switzerland.

Slatkin M (1991) Inbreeding coefficients and coalescence times. *Genetical Research*, **58**, 167–175.

Thatcher RC, Pickard LS (1966) The clerid beetle, *Thanasimus dubius*, as a predator of the southern pine beetle. *Journal of Economic Entomology*, **59**, 955–957.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The CLUSTAL_X Windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research*, **24**, 4876–4882.

Vité JP, Williamson DL (1970) *Thanasimus dubius*: prey perception. *Journal of Insect Physiology*, **106**, 233–239.

This study represents the culmination of Natalie Schrey's master's thesis investigating the population genetics of *Thanasimus dubius*. Natalie is currently a PhD student researching the population genetics of the southern pine beetle, *Dendroctonus frontalis*, using microsatellites in the Department of Zoology at Southern Illinois University. John D. Reeve is an Associate Professor in the Department of Zoology at Southern Illinois University. His research interests include predator-prey interactions, models of insect dispersal, and quantitative ecology. Frank Anderson is an Associate Professor in the Department of Zoology at Southern Illinois University. His primary research interests are molluscan phylogeny and population genetics, with particular focus on land snails and cephalopods.
