Evidence for divergence in cuticular hydrocarbon sex pheromone between California and Mississippi (United States of America) populations of bark beetle parasitoid *Roptrocerus xylophagorum* (Hymenoptera: Pteromalidae)

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Abstract—*Roptrocerus xylophagorum* (Ratzeburg) (Hymenoptera: Pteromalidae) is a common Holarctic parasitoid of the larvae and pupae of bark beetles (Coleoptera: Curculionidae: Scotytinae). In no-choice laboratory bioassays, we found that male wasps derived either from northern California or southwestern Mississippi, United States of America more frequently displayed sexual behaviours (including mounting, wing fanning, and copulation attempts) to glass bulb decoys treated with hexane cuticular washes of females derived from the same parasitoid population rather than the distant population. This result suggests that the composition of the cuticular hydrocarbon sex pheromone has diverged between eastern and western populations and is consistent with previous data indicating that *R. xylophagorum* may consist of more than one species.

The cuticles of insects are coated with complex and often taxonomically specific blends of long-chained hydrocarbons that can play important roles in insect chemical communication (Singer 1998). These cuticular hydrocarbons function as contact pheromones used in mate recognition by at least some parasitic Hymenoptera (Ruther *et al.* 2011; Stökl *et al.* 2014). However, no studies to date have investigated geographic variation in cuticular hydrocarbon sex pheromones within a species of parasitic wasp. *Roptrocerus xylophagorum* (Ratzeburg) (Hymenoptera: Pteromalidae) is a generalist ectoparasitoid of the larvae and pupae of certain species of bark beetles (Coleoptera: Curculionidae: Scolytinae) and is native to both North America and Europe (Bushing 1965; Mills 1983). Reproductive behaviour by male *R. xylophagorum* is triggered by contact with or close proximity to a cuticular hydrocarbon sex pheromone from females (Sullivan 2002). When applied to a solvent-washed female cadaver or a female-sized glass bulb, female cuticular extracts elicit a sequence of stereotyped behaviours from the male, which include mounting of the object, wing-fanning, antennal palpation, extrusion of the genitalia, and attempts to copulate (Sullivan 2002). Evidence from morphological studies and cursory crossing experiments indicates that *R. xylophagorum* from California and Georgia, United States of America may consist of two distinct species (Samson 1984). We conducted the following experiment to determine whether males of *R. xylophagorum* derived from either its western (California) or southeastern (Mississippi) ranges in the United States of America could discriminate the cuticular hydrocarbon sex pheromones of females from these respective regions.

Roptrocerus xylophagorum for experiments were derived from populations at either Blodgett Experimental Forest in northern California

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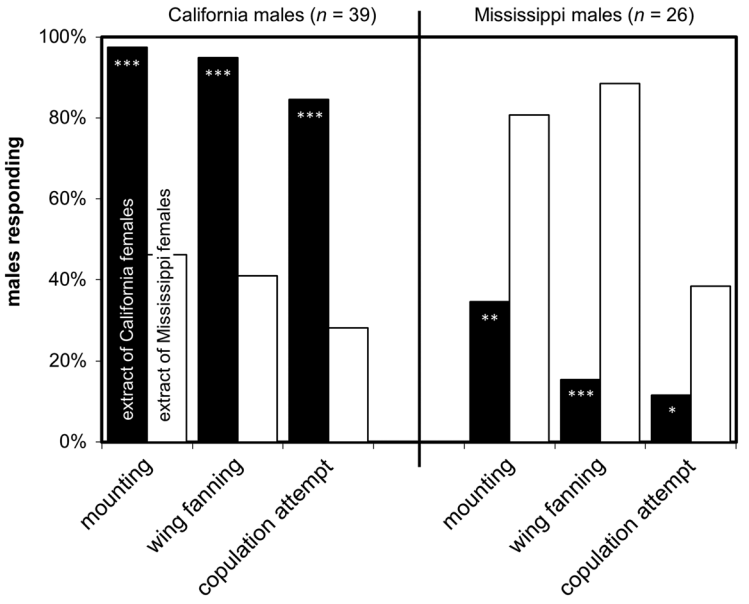
(38°54'N, 120°40'W) or the Homochitto National Forest in western Mississippi (31°25'N, 90°59'W). California *R. xylophagorum* were reared from bolts of *Pinus ponderosae* Lawson and Lawson (Pinaceae) infested in the laboratory with *Ips paraconfusus* Lanier (Coleoptera; Curculionidae) and exposed to ovipositing female *R. xylophagorum*. These *R. xylophagorum* parents had been reared from bolts of *P. ponderosae* that had been naturally infested in the field by *I. paraconfusus* and contained parasitised beetle brood. Mississippi *R. xylophagorum* were reared from bolts of *Pinus taeda* Linnaeus naturally infested in the field by *Dendroctonus frontalis* Zimmermann (Coleoptera; Curculionidae) and various species of *Ips* De Geer (Coleoptera; Curculionidae). California and Mississippi wasps were collected every one to three days as they emerged from bolts confined within rearing enclosures (located at laboratories in Berkeley, California, United States of America and Pineville, Louisiana, United States of America, respectively). Collected wasps were housed in foam rubber-stoppered Erlenmeyer flasks (250 mL) provisioned with honey and water and were used in experiments 6–17 days later. Within five days of collection, the California parasitoids were shipped overnight chilled with ice to the Pineville laboratory for experiments. Flasks from both sites were generally maintained at 4–8 °C to enhance the longevity of the wasps. To obtain cuticular hydrocarbon extracts, females were killed in a −80 °C freezer and then steeped in groups of 20 in 2 mL redistilled hexane (to give 0.1 female equivalents per 10 µL extract) for 15 minutes at room temperature with occasional gentle shaking. Ten microliters of extract of females of either population were applied with a glass pipette to glass bulb decoys (3–4-mm long × 1.5-mm diameter; Sullivan 2002). Decoys were allowed to dry and stored at room temperature for two to three days prior to trials. Males were isolated from females into acetone-rinsed Erlenmeyer flasks provisioned with honey and water and maintained at room temperature one to three days prior to bioassays. For each trial, a single male was confined into a glass tube (15 long × 5-mm interior diameter) into which the glass decoy was suspended on the point of an insect pin. The male was observed under a dissecting microscope for two minutes and we recorded whether the male (1) mounted the decoy, (2) fanned its wings, and (3) attempted to copulate. Males from either location were tested with decoys treated with female extract from either location (*i.e.*, all four possible combinations), with individual males being tested twice (once with either extract in random order but on different days). The treatment order was randomised with equal numbers of tests of each extract with either California or Mississippi males on each test day. Decoys were used in four different trials (all with males of the same origin) and then replaced with new ones. Tests were performed at room temperature at 0930–1800 hours on 29 September to 19 October 2002. For each of behaviours 1–3, the proportions of responses by males from each location to either female extract were compared by a Fisher exact test. Because males were isolated from females and associated semiochemicals for one day between trials with either extract, the two trials with each male were treated as independent replicates (five to nine hours isolation from females is apparently sufficient for males to recover full responsiveness following exposure to female pheromone; Sullivan 2002).

California-derived *R. xylophagorum* males mounted, fanned their wings, and attempted copulation with significantly greater frequency in response to decoys treated with extract of California females rather than Mississippi females; Mississippi males displayed the reciprocal response (Fig. 1). The result shows that the cuticular hydrocarbon sex pheromone of *R. xylophagorum* differs between populations sampled in California and Mississippi, with males showing a clear preference for the pheromone produced by females of the same population. Previously identified differences in the composition of the cuticular hydrocarbon blend of *R. xylophagorum* from the western United States of America (California) and the southeastern United States of America (Georgia) could presumably be mediating male discrimination of the extracts (Espelie *et al.* 1996; Jennings *et al.* 2014). Attempts to cross *R. xylophagorum* derived from these same two areas failed to produce female brood (*i.e.*, evidence of fertilisation; Samson 1984). In combination with evidence of divergence in cuticular hydrocarbon composition (Espelie *et al.* 1996) and the sex pheromone (this study), these data show that eastern and western *R. xylophagorum* may have split or are splitting into different species. A largely host-free zone

Fig. 1. Responses of *Roptrocerus xylophagorum* males derived from populations in either northern California or western Mississippi to glass female decoys treated with hexane extract (0.1 insect equivalents) of the cuticles of females from either population. Fisher exact test contrasts were performed within behaviour type and origin of male (***$P \leqslant 0.001$; **$P \leqslant 0.01$; *$P \leqslant 0.05$).



spanning the Great Plains (Bushing 1965; Wood 1982) likely prevents significant gene flow between eastern and western populations and thus enhances the possibility of genetic differentiation and speciation. Our observations of both (1) attempts by male *R. xylophagorum* from either California or Mississippi to copulate with living females from the other population and (2) male responses to the pheromone blend of the distant population (Fig. 1) indicate that the pheromone differences would not alone be a sufficient reproductive isolation mechanism were the populations to come into contact.

Geographic variation in pheromone composition within an insect species and within continuous distributions of single species is not uncommon (Piston and Lanier 1974; Groot *et al.* 2009; Dyer *et al.* 2014). Furthermore, it is possible for cuticular hydrocarbon compositions of insects to vary due to diet and environmental temperatures, and this may alter the composition of the sex pheromone produced by the female (Fedina *et al.* 2012; Ingleby *et al.* 2014). Thus, geography-associated differences in the composition of a cuticular hydrocarbon pheromone may not necessarily be heritable or evidence of incipient speciation.

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References

Bushing, R.W. 1965. A synoptic list of the parasites of Scolytidae (Coleoptera) in North America north of Mexico. The Canadian Entomologist, **97**: 449–492.

Dyer, K.A., White, B.E., Sztepanacz, J.L., Bewick, E.R., and Rundle, H.D. 2014. Reproductive character displacement of epicuticular compounds and their contribution to mate choice in *Drosophila subquinaria* and *Drosophila recens*. Evolution, **68**: 1163–1175.

Espelie, K.E., Berisford, C.W., and Dahlsten, D.L. 1996. Use of cuticular hydrocarbons in bark beetle parasitoid taxonomy: a study of *Roptrocerus xylophagorum* (Ratzeburg) (Hymenoptera: Torymidae) from the United States, Europe and Australia. Comparative Biochemistry and Physiology (B), **113**: 193–198.

Fedina, T.Y., Kuo, T.H., Dreisewerd, K., Dierick, H.A., and Yew, J.Y. 2012. Dietary effects on cuticular hydrocarbons and sexual attractiveness in *Drosophila*. Public Library of Science One, **7**: e49799.

Groot, A.T., Inglis, O., Bowdridge, S., Santangelo, R.G., Blanco, C., and Lopez, J. 2009. Geographic and temporal variation in moth chemical communication. Evolution, **63**: 1987–2003.

Ingleby, F.C., Hosken, D.J., Flowers, K., Hawkes, M.F., Lane, S.M., Rapkin, J., *et al*. 2014. Environmental heterogeneity, multivariate sexual selection and genetic constraints on cuticular hydrocarbons in *Drosophila simulans*. Journal of Evolutionary Biology, **27**: 700–713.

Jennings, J.H., Etges, W.J., Schmitt, T., and Hoikkala, A. 2014. Cuticular hydrocarbons of *Drosophila montana*: geographic variation, sexual dimorphism and potential roles as pheromones. Journal of Insect Physiology, **61**: 16–24.

Mills, N.J. 1983. The natural enemies of scolytids infesting conifer bark in Europe in relation to biological control of *Dendroctonus* spp. in Canada. Biocontrol News and Information, **4**: 305–328.

Piston, J.J. and Lanier, G.N. 1974. Pheromones of *Ips pini* (Coleoptera: Scolytidae): response to interpopulational hybrids and relative attractiveness of males boring in two host species. The Canadian Entomologist, **106**: 247–251.

Ruther, J., Döring, M., and Steiner, S. 2011. Cuticular hydrocarbons as contact sex pheromone in the parasitoid *Dibrachys cavus*. Entomologia Experimentalis et Applicata, **140**: 59–68.

Samson, P.R. 1984. The biology of *Roptrocerus xylophagorum* (Hym.: Torymidae), with a note on its taxonomic status. Entomophaga, **29**: 287–298.

Singer, T.L. 1998. Roles of hydrocarbons in the recognition systems of insects. American Zoologist, **38**: 294–405.

Stökl, J., Dandekar, A.T., and Ruther, J. 2014. High chemical diversity in a wasp pheromone: a blend of methyl 6-methylsalicylate, fatty alcohol acetates and cuticular hydrocarbons releases courtship behavior in the drosophila parasitoid *Asobara tabida*. Journal of Chemical Ecology, **40**: 159–168.

Sullivan, B.T. 2002. Evidence for a sex pheromone in the bark beetle parasitoid *Roptrocerus xylophagorum*. Journal of Chemical Ecology, **28**: 1045–1063.

Wood, S.L. 1982. The bark and ambrosia beetles of North and Central America (Coleoptera: Scolytidae), a taxonomic monograph. Great Basin Naturalist Memoirs, **6**: 1–1359.