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Do novel genotypes drive the success of an invasive bark beetle–fungus complex? Implications for potential reinvasion

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Abstract. Novel genotypes often arise during biological invasions, but their role in invasion success has rarely been elucidated. Here we examined the population genetics and behavior of the fungus, *Leptographium procerum*, vectored by a highly invasive bark beetle, *Dendroctonus valens*, to determine whether genetic changes in the fungus contributed to the invasive success of the beetle–fungal complex in China. The fungus was introduced by the beetle from the United States to China, where we identified several novel genotypes using microsatellite markers. These novel genotypes were more pathogenic to Chinese host seedlings than were other genotypes and they also induced the release of higher amounts of 3-carene, the primary host attractant for the beetle vector, from inoculated seedlings. This evidence suggests a possible mechanism, based on the evolution of a novel genotype during the two or three decades since its introduction, for the success of the beetle–fungal complex in its introduced region.

Key words: *bark beetles–ophiostomatoid fungi–hosts interactions; chemical ecology;* Dendroctonus valens; *fungal genetics; invasion mechanism; invasive bark beetles;* Leptographium procerum; *3-carene*.

Introduction

During biological invasions, invading species often experience new selection pressures and can therefore be expected to yield novel genotypes (Tsutsui et al. 2000, Lee 2002). How such novel genotypes facilitate the invasion of the introduced species, however, is poorly understood. The red turpentine beetle (RTB), *Dendroctonus valens* LeConte (Coleoptera: Scolytinae), along with its symbiotic fungus, *Leptographium procerum* (Kendr.) Wingf. (see Plate 1), is highly invasive in China and exhibits different behaviors there than in its native region, North America (Yan et al. 2005). To explore the reasons for these differences, we examined the population genetics and behavior of the fungi vectored by RTB in China and discovered a mechanism, centered on fungal induction of a host monoterpene, that may explain the success of the beetle/fungal complex in the invaded region.

In its native North America, RTB is a secondary colonizer of weakened trees, rarely killing healthy pines (Owen et al. 2010). It was inadvertently introduced into China about two decades ago and has since become an aggressive, tree-killing species causing extensive mortality of over seven million healthy Chinese pines (*Pinus tabuliformis* Carrière; Yan et al. 2005). Lu et al. (2009), using morphological and molecular characters, demonstrated that the dominant symbiotic fungus of RTB in China is *L. procerum*, which is also often associated with the beetle in eastern North America (Wingfield 1983). Certain traits of Chinese strains of *L. procerum*, including the ability to suppress other fungal species, have been implicated in the increased aggressiveness of the beetle–fungus complex in China (Lu et al. 2010), but the relationship of native and introduced *L. procerum* was unclear. To elucidate the mechanisms underlying the success of this fungus–bark beetle complex in the introduced region, we used microsatellites to distinguish novel fungal genotypes arising in China since the introduction from the United States. We then compared various attributes of the novel and native fungal genotypes, including pathogenicity to Chinese pine seedlings, differential induction of host volatiles from host seedlings, and attraction of RTB beetles to the volatiles induced by fungi from host seedlings.

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Methods

Fungal population differentiation, gene/genotype diversity, and genotype groups

We analyzed 96 isolates from the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa, representing three populations of *L. procerum* collected from RTB and other bark beetles in China and the United States (details of DNA extraction, amplification, and GENESCAN analysis are given in Appendix A, and a list of isolates is given in Appendix B: Table B1). We defined the three populations as UR (31 fungal isolates from American RTB), UO (14 fungal isolates from other bark beetles in USA), and CR (51 fungal isolates from Chinese RTB). Gene diversity was determined by allele frequencies at each locus, and gene diversity of each population was calculated using the formula, $H = 1 - \Sigma_k X_k^2$, where X_k is the frequency of the kth genotype (POPGENE version 1.31). Differences in allele frequencies for the eight polymorphic loci across clone-corrected populations were calculated using contingency χ^2 tests. In the clone-corrected data set each genotype in a population was represented only once in order to ensure that estimates of allele frequencies are not biased by over-representation of a few clones. In order to evaluate the level of differentiation between clone-corrected populations, θ values were calculated in MULTILOCUS version 1.2. The θ value is an estimate of the fixation index Fst, using the equation $\theta = Q - q/1 - q$, where Q is the probability that two alleles from the same population are the same, and q is the probability that two alleles from different populations are the same. A value of θ equal to 0 indicates no population differentiation, while a value of θ equal to 1 indicates no shared alleles between two populations. The significance of θ was determined by comparing the observed value to that of 1000 randomized data sets in which individuals were randomized across populations. The null hypothesis, that there is no population differentiation, was rejected when $P \leq 0.05$. Using the microsatellite profiles of isolates, each of the 96 fungal isolates was placed into one of five groups: URDG (21 distinct genotypes from the UR population), URSG (5 shared genotypes from the UR population), UOG (13 genotypes from the UO population), CRDG (24 distinct genotypes from the CR population), and CRSG (5 shared genotypes from the CR population) (see Appendix B [Table B6] for full clarification). For several of the following experiments, just one isolate from each genotype group was used as representative.

Seedling inoculations, pathogenicity tests, and volatile extractions

Sixty-eight isolates of *L. procerum* from the FABI collection were used for inoculations of two-year-old *P. tabuliformis* seedlings (Appendix B: Table B6). The seedlings (6–8 mm diameter stems) were grown for four weeks in a greenhouse at ~25°C. For inoculations, circular cuts were made on the bases of seedlings using a 4 mm diameter cork borer to remove bark and expose the cambium. Plugs of mycelium taken from the growing margin of seven-day-old fungal cultures grown on malt extract agar (MEA) were placed into the wounds with mycelial surface facing the cambium. Plugs of MEA alone (without fungi) were applied to trees to serve as controls. To prevent desiccation and contamination, inoculated wounds were sealed with laboratory film (Parafilm M, Pechiney Plastic Packaging, Chicago, Illinois, USA). After 21 d, 10 seedlings of all 13 inoculated seedlings per treatment were randomly selected and uprooted and the lesion length resulting from inoculation was measured. Re-isolation assays of culture comparisons ruled out any potential cross-contamination.

Monoterpene levels were determined in the 10 seedlings per treatment using methods described fully in Appendix A. Briefly, phloem samples were finely chopped, and then monoterpenes were extracted in 20-mL triangle vials (Kimble Bomex Glass, Beijing, China) with 10 mL hexane for 24 h, with 0.1% *p*-cymene added as an internal standard gas chromatograph–mass spectrometer (GC–MS) analysis.

Three seedlings per treatment were also randomly sampled after 21 d for headspace monoterpene volatiles using an effluvial headspace sampling method to collect volatiles from seedlings. Each potted plant was enclosed in a plastic oven bag (Reynolds, Richmond, Virginia, USA) and sealed with self-sealing strips at the opening around each stem, 1–2 cm above soil level. Purified and humidified compressed air was pushed into the bag at the rate of 200 mL/min, and then drawn from the bag via an in-line monoterpene collector (a glass tube with an internal diameter of 3 mm) containing 100 mg of Super Q (80–100 mesh, Alltech Association, Deerfield, Illinois, USA). Volatile compounds were extracted from the Super-Q using hexane with 0.1% *p*-cymene as internal standard. Collected volatiles were analyzed and identified (details in Appendix A).

The fungal-induced lesion lengths and quantities of α-pinene, camphene, β-pinene, myrcene, 3-carene, limonene, β-phellandrene, and terpinolene from phloem (ng/g phloem within lesion) and headspace (ng/min) among treatments were analyzed for each genotype and each genotype group using one-way ANOVA with the Bonferroni correction for multiple comparisons among the treatments. Since new seedlings were used for each measurement, we assumed all responses to be independent. For ANOVA analyses, we tested the normal distribution (normality diagnostics) and homogeneity (Levene's test) of the variances of the responses. We used SPSS 11.5 (SPSS 2001) for statistical procedures.

Walking responses of RTB to 3-carene

RTB walking responses were assessed in a glass Y-tube olfactometer (35-mm diameter × 40 cm long, with a 120° inside angle) with airflow at 200 mL/min through

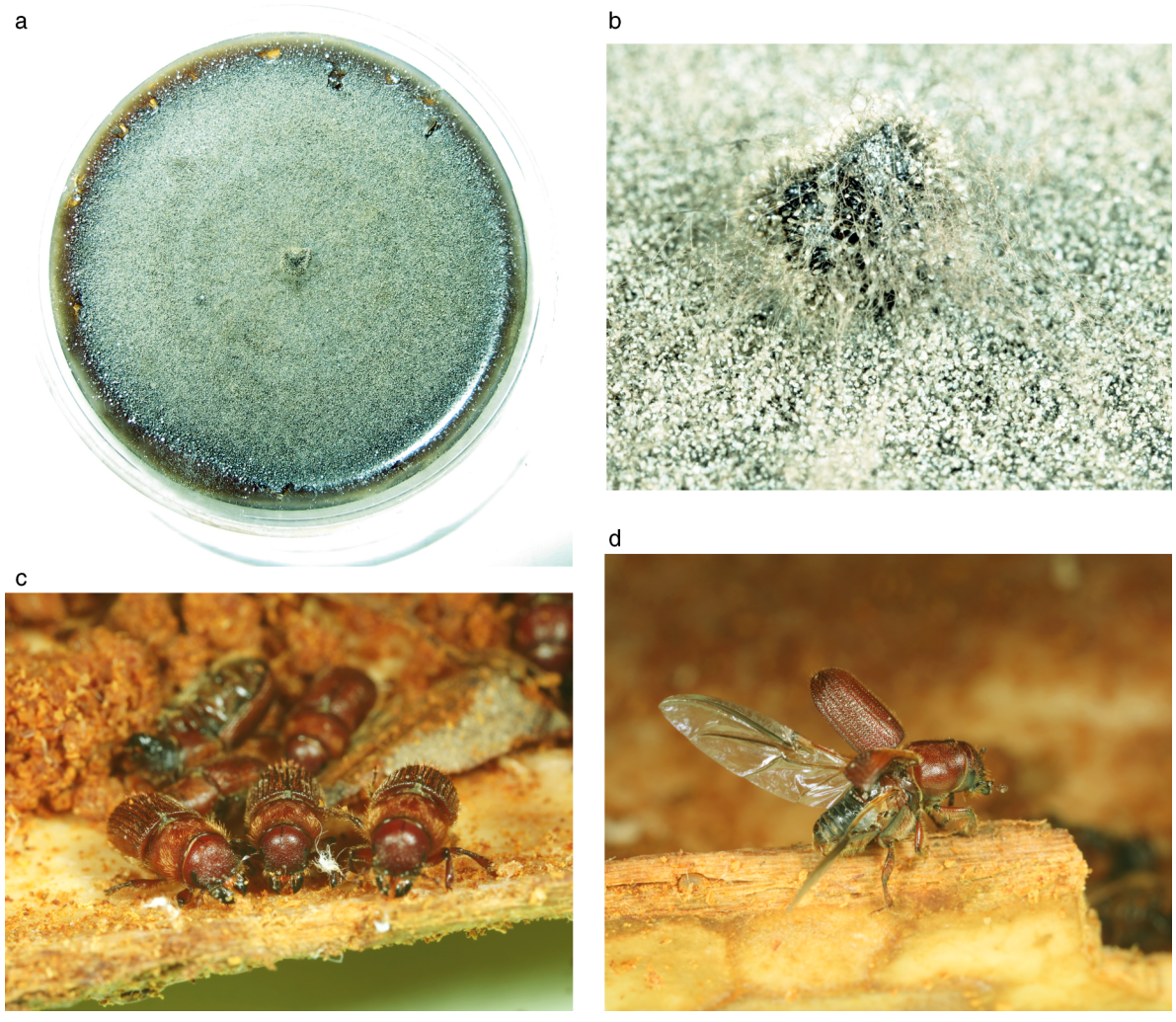

PLATE 1. (a, b) Cultures of *Leptographium procerum*. (c, d) Aggregation of red turpentine beetles and a beetle prior to flight. Photo credit: Runzhi Zhang.

each branch. Incoming air maintained at 25°C and 70% RH was filtered through activated charcoal and humidified then was split between two holding chambers, one serving as a control (solvent blank) and the other chamber holding the test material. Doses of 3-carene (1 ng, 5 ng, 10 ng, 50 ng, 100 ng, 500 ng, and 1000 ng, each in 10 μL hexane) were applied to filter paper strips (5 × 50 mm) that were placed in the holding chamber after the solvent had been allowed to evaporate for 20 s. Air passed from each holding chamber into the respective branches of the Y-tube. A smoke test verified laminar airflow throughout the olfactometer. Thirty minutes before trials began, adult RTB were placed into individual holding tubes and isolated from possible semiochemical sources. At the beginning of each trial, a single beetle (male or female) was placed at the downwind end of the Y-tube, and was given 10 min to respond. The choice of left or right branches of the olfactometer was noted when the beetle walked 5 cm past the Y-tube junction. Treatments associated with right and left branches of the olfactometer were exchanged after every fifth beetle, and Y-tubes were replaced with clean ones when treatments or positions changed. Individual beetles were tested only once. The null hypothesis that beetles showed no preference for either olfactometer arm (and thus showed no response to test compound) was tested using a table of cumulative binomial probabilities with a P value of 0.05. We used SPSS 11.5 (SPSS 2001) for the statistical procedures.

Four isolates of *L. procerum* were used in 2-, 3-, 4-, and 5-yr-old seedling inoculations (Appendix B: Table B6). Inoculations were performed, and then 10 seedlings per treatment per plant age were sampled at 21 d for headspace volatiles. An effluvial headspace sampling method was used to collect volatiles from 2-, 3-, 4-, and 5-yr-old seedlings inoculated with different fungal strains. A two-way ANOVA with treatment, plant age, and treatment × plant age interaction as fixed effects was

TABLE 1. Allele frequencies at eight loci for *Leptographium procerum* populations collected from China and the United States.

Primer and populations	Allele designation										
	A	B	C	D	E	F	G	H	I	J	K
LP1											
CR		0.18	0.08		0.59		0.14	0.02			
UR		0.19	0.06	0.03	0.45		0.13	0.03	0.06	0.03	
UO	0.07	0.50		0.07		0.07			0.21		0.08
LP3											
CR			0.20	0.20	0.31	0.29					
UR		0.03	0.13	0.19	0.35	0.19	0.06			0.03	
UO	0.07	0.14	0.21	0.07		0.07	0.29	0.07	0.07		
LP5											
CR			0.29		0.71						
UR	0.06		0.19	0.10	0.58	0.03	0.03				
UO		0.08	0.50		0.07	0.07	0.28				
LP7											
CR		0.29	0.71								
UR		0.26	0.68	0.06							
UO	0.07	0.43	0.14	0.36							
LP9											
CR				0.27	0.02	0.57	0.14				
UR	0.03		0.13	0.21	0.02	0.42	0.13	0.06			
UO		0.07		0.29	0.21	0.07		0.21	0.14		
LP11											
CR			0.29	0.20	0.51						
UR		0.07	0.19	0.06	0.61	0.07					
UO	0.07	0.21	0.29		0.07	0.36					
LP13											
CR		0.27	0.02	0.71							
UR		0.24	0.02	0.52	0.16	0.06					
UO	0.07	0.50		0.07		0.36					
LP15											
CR		0.06	0.24	0.20	0.50						
UR		0.13	0.13	0.03	0.65	0.03	0.03				
UO	0.07	0.14	0.36		0.07	0.14	0.21				

Note: LP1, LP3, LP5, LP7, LP9, LP11, and LP13 are eight polymorphic fluorescent-labeled primers. Abbreviations are: UR, USA-RTB; UO, USA-other bark beetles; CR, China-RTB (see *Methods: Fungal population differentiation, gene/genotype diversity, and genotype groups* and Appendix B [Table B6] for further clarification of genotype groups).

applied to each of the following responses: eight compounds (α-pinene, camphene, β-pinene, myrcene, 3-carene, limonene, β-phellandrene, and terpinolene) from headspace (ng/g dry seedling, h). Since new seedlings were used for measurement at each occasion, we assumed all the responses to be independent. For all ANOVA analyses, we tested the normal distribution (normality diagnostics) and homogeneity (Levene's test) of the variances of the responses for each treatment. A linear regression model was created for CRDG6 to correlate 3-carene headspace concentrations with plant age. We used SPSS 11.5 (SPSS 2001) for statistical procedures.

RESULTS

The eight pairs of microsatellite markers used to amplify DNA of the 96 isolates yielded 59 alleles in total (Appendix B: Table B3). All the alleles were present in American isolates (UR and UO), but only 29 of them from Chinese isolates (CR) and no private alleles (i.e., alleles unique to that deme) were observed in the introduced population (CR; Appendix B: Table B3). For each microsatellite locus, the alleles of the introduced population vectored by RTB in China (CR population) were all from the native population vectored by the UR population of RTB, and not by other bark beetles (UO population) (Table 1). Furthermore, allele frequencies differed significantly at all eight loci when comparing the UO and CR populations, but there were no significant differences in allele frequencies at any of the eight loci between UR population and CR population (Appendix B: Table B4). Second, θ values showed significant population differentiation between UR and UO populations and between CR and UO populations, but UR and CR populations were almost identical (Appendix B: Table B5).

A total of 68 allele profiles (unique combinations of alleles) were identified among the 96 isolates of *L. procerum* studied. These were distributed across populations as follows: UR (26), UO (13), and CR (29) (Appendix B: Tables B3 and B6). Five different allele profiles among the Chinese isolates had identical microsatellite counterparts in the UR isolates, so we

grouped these isolates with shared allele profiles and coded them as CRSG (Chinese RTB Shared Genotype) and URSG (US RTB Shared Genotype). In other words, URSG1 and CRSG1 had the same allele at each of the eight polymorphic loci, URSG2 and CRSG2 had the same allele at each of the eight polymorphic loci, and so on. Three other allele profile groups were identified and coded as follows: URDG (US RTB Distinct Genotype, meaning that there was no matching allele profile among the Chinese isolates from RTB), UOG (USA-Other bark beetle Genotype), and CRDG (Chinese RTB Distinct Genotype, meaning that there was no matching allele profile among the US isolates from RTB) (Appendix B: Table B6). Hereafter, we refer to the Chinese distinct genotypes as "new" or "novel" because there is no indication of their existence previous to their isolation in China following the introduction of RTB to Asia. The purpose of grouping the allele profiles in this manner was to provide a framework for comparing the behavior of the new Chinese fungal genotypes with that of those from the native region of RTB and *L. procerum*.

Pathogenicity tests showed that the CRDG group (Chinese distinct genotypes) caused significantly longer lesions on seedlings than other groups (Fig. 1a) and more specifically, the lesions caused by CRDG1–2 and CRDG5–24 on the seedlings were significantly longer than those caused by other genotypes (Appendix B: Fig. B1).

The analysis of distilled, extracted volatiles illustrated that the concentrations of 3-carene induced by the Chinese distinct genotypes (CRDG1–2 and CRDG5–24) from inoculated seedlings were significantly higher than those induced by other genotypes (Appendix B: Fig. B2). The Chinese distinct genotype group (CRDG group) induced significantly higher concentrations of 3-carene from the seedlings than other groups by both distilled extraction (Fig. 1b) and headspace extraction (Fig. 2a), demonstrating that these genotypes not only induce higher 3-carene levels in plant tissues, but also cause higher levels of 3-carene release from host trees.

Adult RTB was significantly more attracted to the amounts of 3-carene induced by Chinese distinct genotype (CRDG group) than to levels induced by other fungal groups in the laboratory (Fig. 2b). In addition, the concentrations of 3-carene induced by the fungal genotype CRDG6 representing the Chinese distinct genotype group were positively and significantly correlated with plant age (Appendix B: Fig. B3).

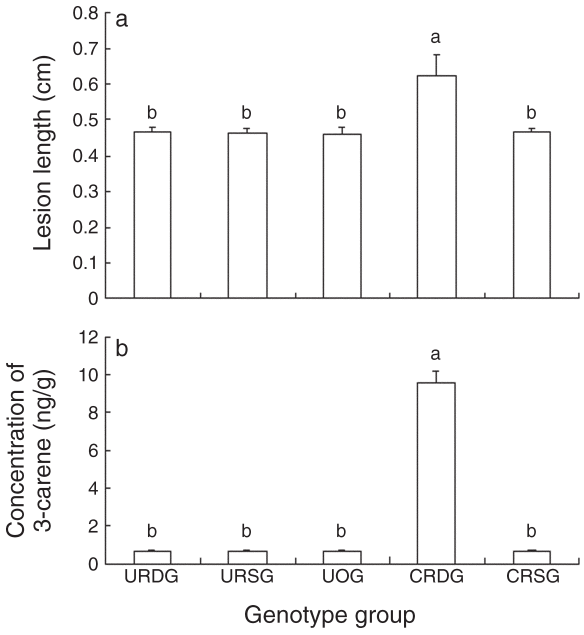

FIG. 1. (a) Lesion lengths and (b) 3-carene concentrations from *Pinus tabuliformis* seedling phloem (mean + SE) associated with inoculations of isolates from URDG, URSG, UOG, CRDG, and CRSG groups after 21 days (see *Methods: Fungal population differentiation, gene/genotype diversity, and genotype groups* and Appendix B [Table B6] for clarification of genotype groups). One-way ANOVA results are $F_{4,63} = 81.67$ ($P < 0.001$) for lesion lengths and $F_{4,63} = 69.12$ ($P < 0.001$) for 3-carene concentrations. Lowercase letters above the bars indicate significance difference at $P < 0.05$.

Discussion

Before the introduction of RTB into China, *L. procerum* had not been reported from that region (Lu et al. 2009), so we assumed that the *L. procerum* inoculum was introduced by RTB from the United States to China as a beetle–fungus complex (Lu et al. 2010). The fact that there were no private alleles in the introduced population supports this hypothesis, as do the identical θ values and allele frequencies in the introduced and native populations. Six and Wingfield (2011) recently suggested that pathogenicity of the fungi vectored by bark beetles could be an important indicator for fungal competition, fungal survival, and resource capture in host trees. The increased lesion lengths caused by the CRDG genotypes suggest that these genotypes may indeed be more pathogenic than the other genotypes (Fig. 1a; Appendix B: Fig. B1). Some studies have shown good correlation between responses from mass-inoculated mature trees and lesion length in wound-inoculated seedlings (Krokene and Solheim 1998, Eckhardt et al. 2004, Lieutier et al. 2004). We have also recently shown a strong correlation between the pathogenicity of *L. procerum* isolates on mature pines with that on seedlings (B. Wang, M. Lu, and J. Sun, *unpublished data*). Thus, our results may indicate that the novel fungal genotypes isolated in China could grow and compete more effectively on Chinese host pines than do the other fungal genotypes.

Increased fungal virulence and induced plant volatiles (the attractants for bark beetle vectors of fungal associates), have both been implicated in the aggressiveness of bark beetles toward hosts (Paine et al. 1997), and previous research suggested that the effect of fungi on host production of monoterpenes might contribute to the success of the beetle–fungal complex in China (Lu et

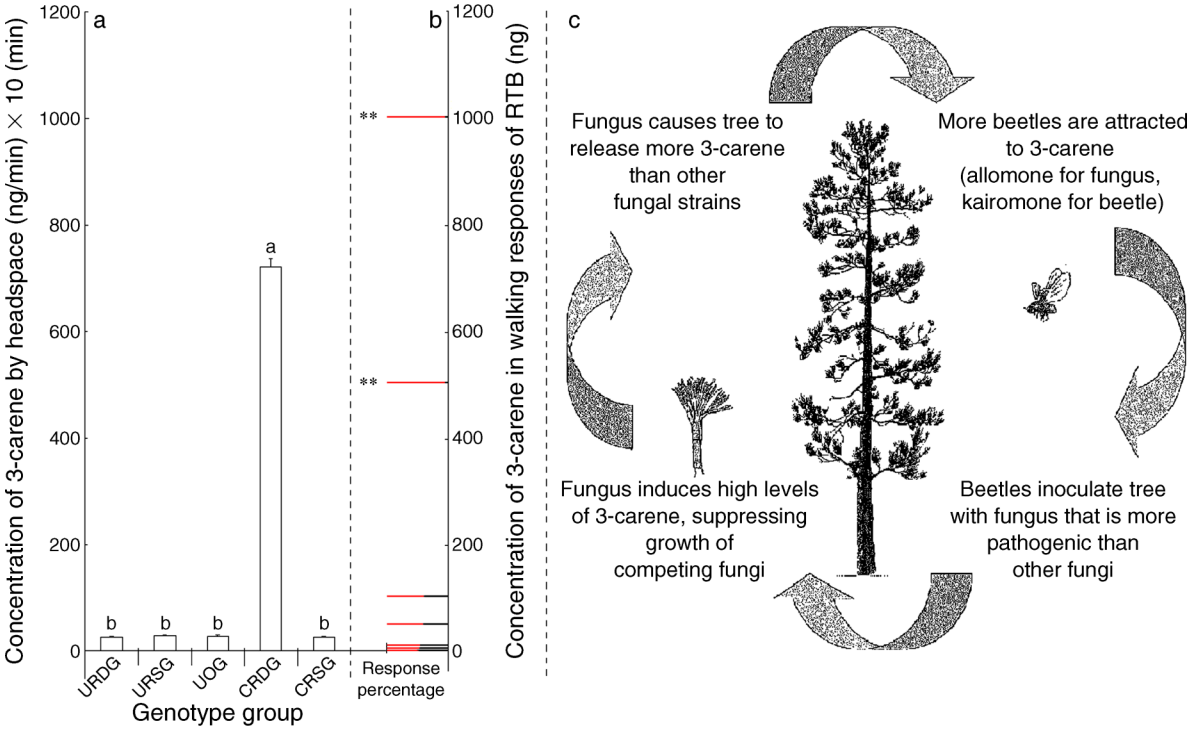

FIG. 2. (a) Headspace concentrations of 3-carene (mean + SE) from *Pinus tabuliformis* seedlings associated with inoculations of *Leptographium procerum* isolates from URDG, URSG, UOG, CRDG, and CRSG groups. One-way ANOVA results for 3-carene headspace concentrations are: $F_{4,63} = 51.66$, $P < 0.001$. Lowercase letters above the bars indicate significant difference at $P < 0.05$. (b) Walking responses of the red turpentine beetle (RTB) to 3-carene at 1, 5, 10, 50, 100, 500, and 1000 ng in Y-tube olfactometer trials. The red lines show the response percentage to 3-carene; dark lines show the response percentage to control ($n = 20$ [10 male, 10 female] responding beetles for each treatment). Total line length (red line + black line) represents 100% response of RTB. (c) Schematic diagram showing beetle–fungus–host tree relationships mediated by induction of 3-carene by novel *L. procerum* genotypes.

** Significant differences at $P < 0.01$.

al. 2010). Our investigation of the role of fungal genotype on production and release of monoterpenes by infected hosts, and of monoterpene release on RTB adults, supports the conjecture that fungal genotype may play a role in monoterpene production and release by infected trees, and consequently on attraction of vector beetles. Previous studies have shown that 3-carene is the most efficient attractant for RTB in China and the United States (Sun et al. 2004, Erbilgin et al. 2007). Here, we have shown that adult RTB was significantly more attracted to the amounts of 3-carene induced by Chinese distinct genotype (CRDG group) than to levels induced by other fungal groups in the laboratory (Fig. 2b). The attraction of RTB by 3-carene was also shown in field tests to be positively related to the release rate of 3-carene at rates ranging from 110 to 210 mg/d (Sun et al. 2004). In addition, the concentrations of 3-carene induced by the fungal genotype CRDG6 representing the novel Chinese genotype group were positively and significantly correlated with plant age (Appendix B: Fig. B3), raising the possibility that introduced novel genotypes can induce even more 3-carene from mature pines in the field, which presumably would attract even more RTB. Thus, the novel genotypes found in China may facilitate aggressiveness of the red turpentine beetle on Chinese host pines (graphically summarized in Fig. 2c).

Recent studies have explored the relationship between genetic variation and success of introduced species (Lee 2002) and had somewhat contradictory findings: Both increased and reduced genetic variation could be cited to explain the success of the introduced species (Tsutsui et al. 2000, Lavergne and Molofsky 2007). Although novel genotypes often occur during biological invasions (Lee 2002), their significance in the success of colonization has rarely been illustrated. Our study demonstrates, perhaps for the first time, that novel fungal genotypes arising during a biological invasion could enhance the ability of the beetle–fungus complex to invade a new ecosystem, and suggests a mechanism to explain the success of the introduced complex. The new alleles that we identified may or may not themselves be causal; we posit, however, that they appear to be good markers for new, more aggressive fungal genotypes and may possibly be integral to sequences that code for proteins that enhance invasive success.

The evolutionary genetics of invasive species can clearly offer insight into mechanisms of invasions (Lee

2002). Our results show that invasion by the beetle–fungus complex was facilitated not by pre-adapted genotypes, but by novel genotypes arising after or during invasion. This finding indicates that successful invasion might depend more on ability to respond to novel selection pressures than on broad plasticity. New hosts in the introduced ranges might have provided such selection pressures for the fungus. Adaptive evolutionary traits that accompanied invasion by this beetle–fungus complex, such as increased fungal pathogenicity toward host pines, are evidence to this point. Thus, evolutionary changes in the fungal population following the introduction of the beetle–fungal complex into a new region might have driven the invasion success of the beetle–fungus complex. Future work, focusing on population genetics and pathogenicity of fungal isolates sampled throughout the native ranges in North America and the introduced ranges in China, should be done to test this inference.

As a symbiotic invasion complex, RTB and its fungal partner displayed several new traits in the introduced range, such as increased aggressiveness of RTB (Yan et al. 2005) and increased virulence and induced host defense chemicals (3-carene) of *L. procerum*. For future studies, it would be interesting to explore whether RTB, together with its newly acquired fungal genotypes, could cause equivalent damage to American pine hosts if it were to reinvade North America. If this were to occur, then the beetle–fungus complex could actually be considered invasive in both the introduced range and the native range. With invasive species, we tend to highlight only the potential danger of the exotic species in the introduced range (Pyšek and Richardson 2010). We suggest that quarantine officials and resource managers should pay special attention to those native beetle–fungus complexes that have become invasive in their introduced ranges, because of their potential to reinvade their native ecosystems carrying more virulent microbial associates.

Acknowledgments

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Literature Cited

Eckhardt, L. G., J. P. Jones, and K. D. Klepzig. 2004. Pathogenicity of *Leptographium* species associated with loblolly pine decline. Plant Disease 88:1174–1178.

Erbilgin, N., S. R. Mori, J. H. Sun, J. D. Stein, D. R. Owen, L. D. Merrill, R. Campos Bolaños, K. F. Raffa, T. Méndez Montiel, D. L. Wood, and N. E. Gillette. 2007. Response to host volatiles by native and introduced populations of *Dendroctonus valens* (Coleoptera: Curculionidae, Scolytinae) in North American and China. Journal of Chemical Ecology 33:131–146.

Krokene, P., and H. Solheim. 1998. Assessing the virulence of four bark beetle-associated bluestain fungi using Norway spruce seedlings. Plant Pathology 47:537–540.

Lavergne, S., and J. Molofsky. 2007. Increased genetic variation and evolutionary potential drive the success of an invasive grass. Proceedings of the National Academy of Sciences USA 104:3883–3888.

Lee, C. E. 2002. Evolutionary genetics of invasive species. Trends in Ecology and Evolution 17:386–391.

Lieutier, F., A. Yart, D. Sauvard, and V. Gallois. 2004. Variations in growth and virulence of *Leptographium wingfieldii* Morelet, a fungus associated with the bark beetle *Tomicus piniperda* L. Annals of Forest Science 61:45–53.

Lu, M., M. J. Wingfield, N. E. Gillette, S. R. Mori, and J.-H. Sun. 2010. Complex interactions among host pines and fungi vectored by an invasive bark beetle. New Phytologist 187:859–866.

Lu, M., X. D. Zhou, Z. W. De Beer, M. J. Wingfield, and J.-H. Sun. 2009. Ophiostomatoid fungi associated with the invasive pine-infesting bark beetle, *Dendroctonus valens*, in China. Fungal Diversity 38:133–145.

Owen, D. R., S. L. Smith, and S. J. Seybold. 2010. Red turpentine beetle. Forest insect and disease leaflet number 55. USDA Forest Service, Pacific Northwest Region, Portland, Oregon, USA.

Paine, T. D., K. F. Raffa, and T. C. Harrington. 1997. Interactions among scolytid bark beetles, their associated fungi, and live host conifers. Annual Review of Entomology 42:179–206.

Pyšek, P., and D. M. Richardson. 2010. Invasive species, environmental change and management, and health. Annual Review of Environment and Resources 35:25–55.

Six, D. L., and M. J. Wingfield. 2011. The role of phytopathogenicity in bark beetle-fungal symbioses: A challenge to the classic paradigm. Annual Review of Entomology 56:255–272.

SPSS. 2001. SPSS. Version 11.5. SPSS, Chicago, Illinois, USA.

Sun, J., Z. Miao, Z. Zhang, and N. Gillette. 2004. Red turpentine beetle, *Dendroctonus valens* LeConte (Coleoptera: Scolytidae), response to host semiochemicals in China. Environmental Entomology 33:206–212.

Tsutsui, N. D., A. V. Suarez, D. A. Holway, and T. J. Case. 2000. Reduced genetic variation and the success of an invasive species. Proceedings of the National Academy of Sciences USA 97:5948–5953.

Wingfield, M. J. 1983. Association of *Verticicladiella procera* and *Leptographium terrebrantis* with insects in the Lake States. Canadian Journal of Forest Research 13:1238–1245.

Yan, Z., J. Sun, D. Owen, and Z. Zhang. 2005. The red turpentine beetle, *Dendroctonus valens* LeConte (Scolytidae): an exotic invasive pest of pine in China. Biodiversity and Conservation 14:1735–1760.

APPENDIX A

Detailed methods for "novel fungal genotypes" (*Ecological Archives* E092-175-A1).

APPENDIX B

Tables and figures for "novel fungal genotypes" *Ecological Archives* E092-175-A2).