



United States
Department
of Agriculture
Forest Service

Intermountain
Research Station

Research Note
INT-RN-424

February 1995



The Effect of Phloem Thickness on Heterozygosity in Laboratory- Reared Mountain Pine Beetles

Gene D. Amman
Molly W. Stock

Abstract—Mountain pine beetles (*Dendroctonus ponderosae* Hopkins) were collected from naturally infested trees of lodgepole pine (*Pinus contorta* Dougl.) in northern Utah. Beetles were reared in logs through six generations in a laboratory, and heterozygosity measured. Heterozygosity levels initially decreased when individual pairs of beetles were reared. However, when beetles were allowed to select mates at random, heterozygosity rose to levels higher than those in the starting population. Heterozygosity was higher in beetles reared in thin than those reared in thick phloem.

Keywords: Coleoptera, *Dendroctonus ponderosae*, electrophoresis, genetic variability, Scolytidae

Outbreaks of the mountain pine beetle (*Dendroctonus ponderosae* Hopkins) cause widespread loss of pines, especially lodgepole pine (*Pinus contorta* Dougl.) in western Canada (Safranyik and others 1974) and the Western United States (Amman and others 1977). During epidemics, a single National Forest may lose in excess of a million trees in 1 year; for example, 3.6 million lodgepole pine trees were killed on the Targhee National Forest, ID, in 1976 (Klein and others 1979). Individual lodgepole stands may have 70 to 94 percent of the trees larger than 12.7 cm in diameter killed during the course of an infestation (McGregor and others 1987).

As a result, factors influencing population fluctuations in this insect have received a great deal of attention. Several factors are believed to have important roles in regulating populations. These have

centered around host resistance (Raffa and Berryman 1982; Shrimpton 1978), food availability (Amman 1972; Cole and others 1976), and weather (Bentz and others 1991; Safranyik 1978).

Natural populations contain high levels of genetic variation, and studies of this variation have provided insight not attainable by other techniques into the responses of a population to environmental factors. It is well established that heterozygosity in a population confers greater fitness in a natural, varying environment. Heterozygous populations are often able to maintain larger population sizes or biomass than less heterozygous populations (Beardmore 1983). These advantageous effects can, in most cases, be attributed to heterozygosity, not to the effects of specific gene combinations (Mitton and Grant 1984).

The advantage of heterozygosity is frequently expressed as higher survival under severe or stressful environmental conditions (Bryant 1974, 1976; Milkman 1978; Parson 1971, 1987; Samollow and Soule 1983; Smith and others 1975). Mountain pine beetles emerging from thin-phloem lodgepole pine were more heterozygous than beetles collected from thick-phloem lodgepole pine (Stock and Amman 1985), and overwintered pine engraver beetles (*Ips pini*) were significantly more heterozygous than their nonoverwintered counterparts (Gast and Stock, in press). Heterozygosity changes observed over time in several mountain pine beetle populations in the field corresponded with density fluctuations and, presumably, the environmental stressors that influence population numbers (Stock and others, in preparation).

Laboratory studies with *Drosophila* and other organisms have helped elucidate the effect of selective agents on gene polymorphism at individual gene loci (Birley and Beardmore 1977). It seems likely that changes in heterozygosity could be measured, under controlled laboratory conditions, to evaluate the effects of specific selection pressures on a mountain

Gene D. Amman (retired) was Project Leader, Mountain Pine Beetle Research Work Unit, Intermountain Research Station, Forest Service, U.S. Department of Agriculture, Ogden, UT. Molly W. Stock is Professor, Department of Forest Resources, University of Idaho, Moscow.

pine beetle population and to help determine the relative contribution of these factors to population fluctuations in the field.

The work described here focuses on the association between food availability and heterozygosity. Beetles in areas with healthy, unstressed trees with a thick phloem layer have more food and more space to produce galleries and offspring. Beetles attacking trees with thin phloem have less food and produce fewer offspring. We would expect beetles reared in thin phloem to be more heterozygous than beetles reared in thick phloem under similar conditions. In this study, we monitored levels of heterozygosity over six consecutive generations of mountain pine beetle reared in thick and thin phloem in the laboratory.

Methods

Mountain pine beetles were obtained from logs cut on April 17, 1984, from four lodgepole pine trees that had thick (>2.5 mm) phloem. The trees were located along the highway between Greendale Junction (close to Flaming Gorge) and Vernal, in northeastern Utah. This infestation was in epidemic status and near its peak in 1984. Green logs from live trees, used for rearing beetles in the laboratory, were obtained from the same area and were selected on the basis of phloem thickness. Phloem thickness averaged 3 mm on thick-phloem logs and 1.9 mm on thin-phloem logs.

At the Intermountain Research Station's Ogden, UT, laboratory, the infested logs from the field were placed in rearing cages at room temperature (20 to 27 °C). The overwintered larvae completed development and adults started to emerge on May 3, 1984. These adults were collected for genetic analysis for production of the next generation.

Beetles were sexed using characteristics of the seventh abdominal tergum (Lyon 1958). Pairs were introduced into predrilled holes 3 mm in diameter and 25 mm deep made in the phloem at the xylem interface. These holes were spaced 5 cm apart around the basal circumference of the green logs. After the female, followed by the male, was introduced into a hole, a 2.5 cm square piece of wire screen was stapled over the hole to keep the beetles from leaving. These newly infested logs were placed in constant temperature cabinets at 25 °C where beetles made egg galleries and oviposited, and the new generation developed.

When adult beetles of the new generation started to emerge, the logs were placed in individual rearing cages at room temperature. Beetles were collected as they emerged and kept separate by log so that mates could be selected from different logs, thus preventing mating of siblings. This procedure was followed for the F1 and F2 generations. Starting with

emergence of the F2 generation, we placed green logs in the bottom of the temperature cabinets and allowed beetles to infest and mate at random.

Logs were introduced into the cabinet one at a time and left until the amount of boring frass suggested a fairly high attack density had been achieved. After a log became infested it was placed in a rearing cage at a constant 25 °C until adult emergence. This procedure was followed for the F3 through F6 generations.

Live samples of approximately 100 beetles from the original (base) population and from each subsequent generation reared in thick and thin phloem were mailed in petri dishes containing damp sawdust to the University of Idaho laboratory for electrophoretic analysis. Methods for electrophoresis of mountain pine beetles have been described by Higby and Stock (1982) and Bentz and Stock (1986). Within groups, genotype frequencies were compared to values derived from random-mating (Hardy-Weinberg) expectations using a chi-square test. To compare levels of heterozygosity between groups, Nei's (1975) average heterozygosity (H) was calculated and compared with two-tailed t-tests on transformed data.

Results and Discussion

Initially, we assayed a number of loci described in earlier studies of the mountain pine beetle, but, after the second generation, we focused our attention on the six loci (AAT, EST2, EST4, PEP-1a, PEP-gl, and PGI) that were polymorphic in the base population or the F1 generation (table 1). Of these six loci, the esterase locus (EST2) was most polymorphic, and the phosphoglucose isomerase locus (PGI) least polymorphic. Average heterozygosity over the six loci varied from 20 to 36 percent with high values reflecting use of polymorphic loci only. (When monomorphic loci are included in the calculation, heterozygosity in these and related bark beetles averages about 17 percent; Bentz and Stock 1986, Cane and others 1990.)

Figure 1 shows changes observed in average heterozygosity over six generations in the laboratory. Average heterozygosity decreased over the first two generations, with beetles from thin phloem becoming less heterozygous than those from thick phloem. Although this decrease in overall heterozygosity was statistically significant, the number of homozygotes was significantly higher than expected ($P < 0.05$) at the AAT locus in F2 beetles from thick phloem and at both the AAT and EST2 loci in F2 beetles from thin phloem. This led us to suspect that our method of pairing beetles and introducing them into the logs was at least partly responsible for this decline in heterozygosity. For the F1 and F2 generations, 81 to

Table 1—Allele frequencies (n) heterozygosity (h), and average heterozygosity (H) for six consecutive generations (F1-F6) of laboratory-reared mountain pine beetle.

Locus	Base population	F1		F2		F3		F4		F5		F6	
		Thn	Thk	Thn	Thk	Thn	Thk	Thn	Thk	Thn	Thk	Thn	Thk
AAT 1	.77	.83	.74	.82	.76	.64	.61	.65	.68	.58	.62	.61	.55
	.23	.17	.26	.18	.24	.36	.39	.35	.32	.42	.38	.39	.45
N	114	95	93	39	60	109	111	95	104	107	109	90	87
h	.35	.28	.38	.29	.36	.46	.48	.45	.43	.49	.47	.48	.49
EST 1	.03	.03	.03	.02	.03	.05	.03	.04	.07	.05	.07	.07	.05
	.34	.36	.33	.38	.48	.29	.58	.41	.31	.37	.39	.31	.35
	.26	.26	.24	.38	.28	.27	.15	.27	.26	.32	.31	.24	.20
	.12	.13	.11	—	.04	.06	.12	.09	.08	.10	.06	.15	.14
	.23	.21	.27	.22	.16	.33	.10	.19	.28	.15	.16	.21	.24
	.01	.01	.01	.01	.01	—	.02	—	.01	.01	.01	.01	.02
N	112	99	92	44	61	115	119	105	106	108	105	99	98
h	.75	.74	.75	.66	.66	.73	.61	.71	.75	.73	.72	.77	.76
EST 4	.99	.99	1.0	.90	.97	1.0	.98	.99	.98	.99	.99	.98	1.0
	.01	.01	—	.10	.03	—	.02	.01	.02	.01	.01	.02	—
N	118	100	100	44	61	120	120	110	110	120	120	100	100
h	.02	.02	0	.18	.06	0	.04	.02	.04	.02	.02	.04	0
PEP gl	.91	.95	.94	.99	.94	.94	.89	.88	.91	.75	.99	.84	.91
	.09	.05	.06	.01	.06	.07	.12	.12	.09	.25	.10	.16	.09
N	115	100	98	44	61	100	100	109	110	120	120	97	94
h	.16	.09	.11	.02	.11	.11	.19	.21	.16	.37	.02	.27	.16
PEP la	.02	.01	—	.01	.03	—	—	.01	—	.02	—	.25	.01
	.88	.91	.93	.99	.93	.94	.89	.91	.92	.67	.63	.38	.80
	.11	.07	.06	.01	.07	.04	.10	.09	.06	.33	.35	.38	.19
N	115	90	90	44	60	116	113	106	109	86	89	97	98
h	.20	.17	.13	.02	.13	.11	.19	.16	.15	.44	.48	.65	.32
PGI 1	.01	.99	1.0	.99	1.0	1.0	1.0	1.0	.99	1.0	1.0	.99	1.0
	.01	.01	—	.01	—	—	—	—	.01	—	—	.01	—
N	109	100	100	44	61	120	120	110	110	120	120	100	100
h	0	.02	0	.02	0	0	0	0	.02	.02	0	0	0
H	.25	.22	.23	.20	.22	.24	.25	.26	.26	.34	.28	.36	.29

125 handpicked pairs of beetles were introduced into fresh logs. Males and females were selected from different source logs in an attempt to maximize recombination and minimize inbreeding. Nevertheless, the observed decrease in heterozygosity and the low number of galleries produced (only seven in thin phloem and 31 in thick phloem for the F2 generation) indicated that inbreeding was nevertheless occurring and that a change in rearing methods was needed.

After the second (F2) generation, when beetles were permitted to pair up naturally and larger numbers of galleries were subsequently produced, average heterozygosity began to increase rapidly in both groups and continued to increase until the end of the study. Over this period, no significant deviation from Hardy-Weinberg expectations were observed. By the F4 generation, heterozygosity in both groups was slightly higher than in the original population.

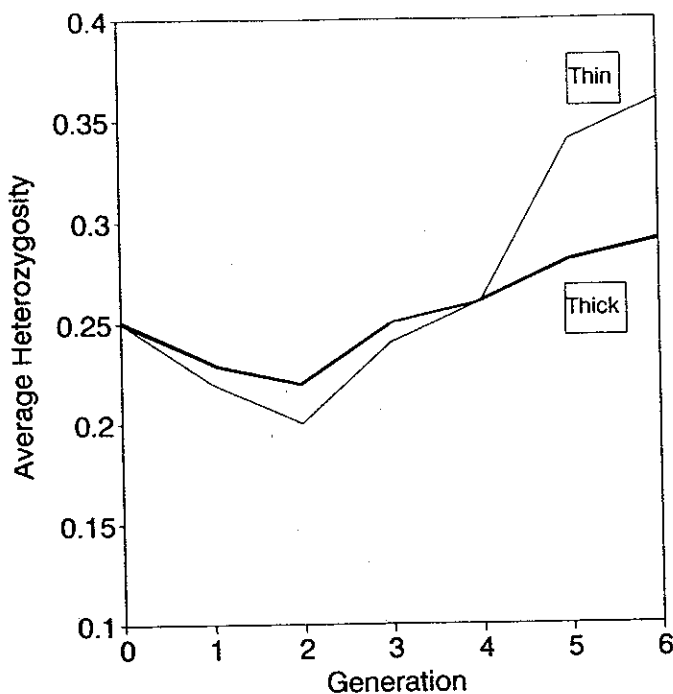


Figure 1—Average mountain pine beetle heterozygosity over six loci generations in laboratory experiments.

During the last four generations, heterozygosity in beetles from thin phloem increased faster than heterozygosity in beetles from thick phloem. At the end of the study, the population from thin phloem was significantly more heterozygous than the population from thick phloem (.36 vs. .29, $P < .01$), and significantly more heterozygous than the base population (.36 vs. .25, also $P < .01$). Although the F6 beetles from thick phloem were more heterozygous than the base population (.29 vs. .25), this difference was not significant.

Overall, the results of this work supported our expectation that heterozygosity would increase over time in beetle populations reared in the less hospitable environment of logs with thin phloem. The results also suggest ways that mountain pine beetle rearing methods might be improved for experiments of this type. This work provides a basis for further laboratory experiments to evaluate the relative contribution of phloem thickness and other variables believed to affect survival and, consequently, population numbers in the mountain pine beetle.

Acknowledgements

We thank Mike Perez for help in rearing beetles and Sandra Gast Kegley for assistance with the laboratory work and computer analyses. Critical

reviews of the manuscript were provided by Barbara Bentz (Intermountain Research Station, Forest Service, U.S. Department of Agriculture), Ken Hobson (Utah State University), and Ken Raffa (University of Wisconsin).

References

- Amman, G. D. 1972. Mountain pine beetle brood production in relation to thickness of lodgepole pine phloem. *Journal of Economic Entomology*. 65: 138-140.
- Amman, G. D.; McGregor, M. D.; Cahill, D. B.; Klein, W. H. 1977. Guidelines for reducing losses of lodgepole pine to the mountain pine beetle in unmanaged stands in the Rocky Mountains. Gen. Tech. Rep. INT-36. Ogden, UT: U.S. Department of Agriculture, Forest Service, Intermountain Forest and Range Experiment Station. 19 p.
- Beardmore, J. A. 1983. Extinction, survival, and genetic variation. In: Schoenewald-Cox, C. M.; Chambers, S. M.; MacBryde, B.; Thomas, W. L., eds. *Genetics and conservation*. The Benjamin/Cummings Publishing Company, Inc., Menlo Park, CA. 727: 125-151 p.
- Bentz, B. J.; Logan, J. A.; Amman, G. D. 1991. Temperature-dependent development of the mountain pine beetle (Coleoptera:Scolytidae) and simulation of its phenology. *Canadian Entomologist*. 123: 1083-1094.
- Bentz, B. J.; Stock, M. W. 1986. Phenetic and phylogenetic relationships among ten species of *Dendroctonus* bark beetles (Coleoptera:Scolytidae). *Annals of the Entomological Society of America*. 79: 527-534.
- Birley, A. J.; Beardmore, J. A. 1977. Genetical composition, temperature, density and selection in an enzyme polymorphism. *Heredity*. 39(1): 133-144.
- Bryant, E. H. 1974. On the adaptive significance of enzyme polymorphisms in relation to environmental variability. *American Naturalist*. 108: 1-19.
- Bryant, E. H. 1976. A comment on the role of environmental variation in maintaining polymorphisms in natural populations. *Evolution*. 30: 188-189.
- Cane, J. H.; Stock, M. W.; Wood, D. L.; Gast, S. J. 1990. Phylogenetic relationships of *Ips* bark beetles (Coleoptera:Scolytidae): electrophoretic and morphometric analyses of Hopping's Group IX. *Biochemical Systematics and Ecology*. 18(5): 359-368.
- Cole, W. E.; Amman, G. D.; Jensen, C. E. 1976. Mathematical models for the mountain pine beetle-lodgepole pine interaction. *Environmental Entomologist*. 5: 11-19.
- Gast, S. J.; Stock, M. W. [In press]. Genetic diversity in overwintered and non-overwintered *Ips pini* (Say) (Coleoptera:Scolytidae) in Idaho. *Pan-Pacific Entomologist*.

- Higby, P. K.; Stock, M. W. 1982. Genetic relationships between two sibling species of bark beetle (Coleoptera:Scolytidae), Jeffrey pine beetle and mountain pine beetle, in northern California. *Annals of the Entomological Society of America*. 75: 668-674.
- Klein, W. H.; Bennett, D. D.; Young, R. W. 1979. A pilot survey to measure annual mortality of lodgepole pine caused by the mountain pine beetle. Rep.78-4. Ogden, UT: U.S. Department of Agriculture, Forest Service, Intermountain Region and Methods Applications Group. 15 p.
- Lyon, R. L. 1958. A useful secondary sex characteristic in *Dendroctonus* bark beetles. *Canadian Entomologist*. 90: 582-584.
- McGregor, M. D.; Amman, G. D.; Schmitz, R. F.; Oakes, R. D. 1987. Partial cutting lodgepole pine stands to reduce losses to the mountain pine beetle. *Canadian Journal of Forest Research*. 17: 1234-1239.
- Milkman, R. 1978. Selection differentials and selection coefficients. *Genetics*. 88: 391-403.
- Mitton, J. B.; Grant, M. C. 1984. Associations among protein heterozygosity, growth rate, and developmental homeostasis. *Annual Review of Ecological Systematics*. 15: 479-499.
- Nei, M. 1975. Genetic variability in natural populations. In: *Molecular population genetics and evolution*. North Holland Research Monographs, *Frontiers of Biology* 40. New York: American Elsevier Publishing Company, Inc.: 127-174.
- Parsons, P. A. 1971. Extreme-environment heterosis and genetic loads. *Heredity*. 26: 479-483.
- Parsons, P. A. 1987. Evolutionary rates under environmental stress. *Evolutionary Biology*. 21: 311-347.
- Raffa, K. F.; Berryman, A. A. 1982. Physiological differences between lodgepole pines resistant and susceptible to the mountain pine beetle and associated microorganisms. *Environmental Entomologist*. 11: 486-492.
- Safranyik, L. 1978. Effects of climate and weather on mountain pine beetle populations. In: Berryman, A. A.; Amman, G. D.; Stark, R. W., tech. eds. *Theory and practice of mountain pine beetle management in lodgepole pine forests*. Symposium proceedings: April 25-27, 1978, Pullman, WA. Moscow, ID: University of Idaho, Forest, Wildlife and Range Experiment Station: 77-84.
- Safranyik, L.; Shrimpton, D. M.; Whitney, H. S. 1974. Management of lodgepole pine to reduce losses from the mountain pine beetle. Tech. Rep. 1. Victoria, BC: Canadian Forestry Service, Pacific Forest Research Centre, Department of the Environment. 24 p.
- Samollow, P. B.; Soule, M. E. 1983. A case of stress related heterozygote superiority in nature. *Evolution*. 37(3): 646-649.
- Shrimpton, D. M. 1978. Effects of lodgepole pine resistance on mountain pine beetle population. In: Berryman, A. A.; Amman, G. D.; Stark, R. W., tech. eds. *Theory and practice of mountain pine beetle management in lodgepole pine forests*. Symposium proceedings: April 25-27, 1978, Pullman, WA. Moscow, ID: University of Idaho, Forest, Wildlife and Range Experiment Station: 64-76.
- Smith, M. H.; Garten, C. T.; Ramsay, P. E. 1975. Genetic heterozygosity and population dynamics in small mammals. In: Markert, C. L., ed. *Isozymes, genetics, and evolution*. New York: Academic Press: 85-102.
- Stock, M. W.; Amman, G. D. 1985. Host effects on the genetic structure of mountain pine beetle, *Dendroctonus ponderosa*, populations. In: Safranyik, L., ed. *The role of the host in the population dynamics of forest insects*. Banff, Alberta, Canada: 83-95.
- Stock, M. W.; Schmitz, R. F.; Amman, G. D. [In manuscript]. Heterozygosity and density change in mountain pine beetle (*Dendroctonus ponderosae*) populations (Coleoptera:Scolytidae).