Complementary sex determination substantially increases extinction proneness of haplodiploid populations

Amro Zayed* and Laurence Packer

Department of Biology, York University, 4700 Keele Street, Toronto, ON, Canada M3J 1P3

Edited by Charles D. Michener, University of Kansas, Lawrence, KS, and approved May 24, 2005 (received for review March 18, 2005)

The role of genetic factors in extinction is firmly established for diploid organisms, but haplodiploids have been considered immune to genetic load impacts because deleterious alleles are readily purged in haploid males. However, we show that single-locus complementary sex determination ancestral to the haplodiploid Hymenoptera (ants, bees, and wasps) imposes a substantial genetic load through homozygosity at the sex locus that results in the production of inviable or sterile diploid males. Using stochastic modeling, we have discovered that diploid male production (DMP) can initiate a rapid and previously uncharacterized extinction vortex. The extinction rate in haplodiploid populations with DMP is an order of magnitude greater than in its absence under realistic but conservative demographic parameter values. Furthermore, DMP alone can elevate the base extinction risk in haplodiploids by over an order of magnitude higher than that caused by inbreeding depression in threatened diploids. Thus, contrary to previous expectations, haplodiploids are more, rather than less, prone to extinction for genetic reasons. Our findings necessitate a fundamental shift in approaches to the conservation and population biology of these ecologically and economically crucial insects.

diploid male production | haplodiploidy | Hymenoptera | pollinator decline | conservation genetics

Haplodiploid insects such as ants, bees, and wasps are crucial components of terrestrial ecosystems, and their conservation is essential for economic as well as ecological reasons (1–4). Despite the obvious differences that result from their sex-determining mechanism, the conservation genetics of haplodiploids has received very little attention (5) and has been ignored in conservation efforts (6, 7). Inbreeding depression in diploid organisms significantly increases extinction risk (8–11), but faster purging of recessive deleterious mutations in haploid males is believed to render haplodiploids relatively immune to its effects (12–14), theoretically reducing their intrinsic extinction risk, compared with diploids. However, single-locus complementary sex determination (sl-CSD), ancestral in the Hymenoptera, introduces an unusual source of genetic load in small populations (15): the production of inviable or effectively sterile diploid males (DMs) from fertilized eggs homozygous at the sex-determining locus, *csd* (16–19) (Fig. 1).

Large haplodiploid populations can maintain many *csd* alleles (commonly 9–20 alleles; ref. 15) and thus have low levels of DM production (DMP). However, drift in small populations reduces *csd* allelic richness and increases DMP (15). Several studies have documented low levels of *csd* allelic richness (<5 alleles) in both natural and introduced populations (20–24). Because female hymenopterans fertilize their eggs to produce daughters only, the production of DMs effectively increases female mortality, thus reducing the potential for population growth (25) (Fig. 1). Further, DMP also reduces the effective breeding size of haplodiploids, especially in small populations (26). These factors suggest that DMP can increase extinction risk in haplodiploids. In this study, we model the effect of DMP on the extinction dynamics of haplodiploid populations and show that DMP renders haplodiploids substantially more, rather than less, prone to extinction.

Methods

Stochastic Model. Because our goal was to examine the relative contribution of DMP to extinction, we did not model other factors known to increase it (10, 27) (e.g., catastrophes and Allee effects), and thus our analyses underestimate the actual extinction risk found in nature. Further, because haplodiploidy does not result in the complete purging of the mutational load (12, 13), modest levels of inbreeding depression in haplodiploids are both expected and observed (14), further increasing the actual extinction risk experienced by natural haplodiploid populations.

We developed an individual-based Monte Carlo model to simulate solitary haplodiploid populations with and without sl-CSD. Our populations were modeled to grow exponentially at a constant average rate until they reach their carrying capacity (K); when growth stopped (27). Demographic stochasticity in sex ratio followed a binomial distribution around the mean primary sex ratio (27, 28). Environmental and demographic stochasticity in net reproductive output (number of offspring per female) was modeled after a normal distribution (27, 28) with a variance of $V_e = 1$ (29) and $V_d = 6$ (10, 30) × mean net reproductive output (NRO), respectively. NRO represents the net number of offspring produced during the lifetime of each female after taking into account mortality, excluding that due to DMP. The average expected intrinsic growth rate (r) for each population replicate is equivalent to ln NRO/2 (e.g., $r = 0$ when NRO = 2 and $r = 1.61$ when NRO = 10). Populations were started at their carrying capacity with a primary male/female ratio of 1:1. The simulations were projected for 100 generations for 1,000 iterations.

For simulations with sl-CSD, we started each population with the equilibrium number of *csd* alleles found in an effective population size of Ne, given a mutation rate of 10^{-6} per generation (31) and a conservative Ne/N ratio of 20% (10, 24), by using the method outlined in ref. 32. Alleles were randomly assigned to individuals in the population at the beginning of each simulation. We assume that mating is random and that females mate singly, the norm for the Hymenoptera (33). We do not simulate mutation of *csd* alleles, because under the time frame of our simulations, it is highly unlikely that new *csd* alleles will arise. Given the largest possible effective population size modeled ($Ne = 100$), the number of new mutations that is expected to arise at the sex locus over 100 generations is negligible (200 gene copies × 10^{-6} per generation × 100 generations = 0.02 mutation arising during our simulation). Further, we assume that when DMs are effectively sterile, they are capable of mating, but

This paper was submitted directly (Track II) to the PNAS office.

Abbreviations: sl-CSD, single-locus complementary sex determination; DM, diploid male; DMP, DM production; NRO, net reproductive output.

*To whom correspondence should be addressed. E-mail: azayed@yorku.ca.

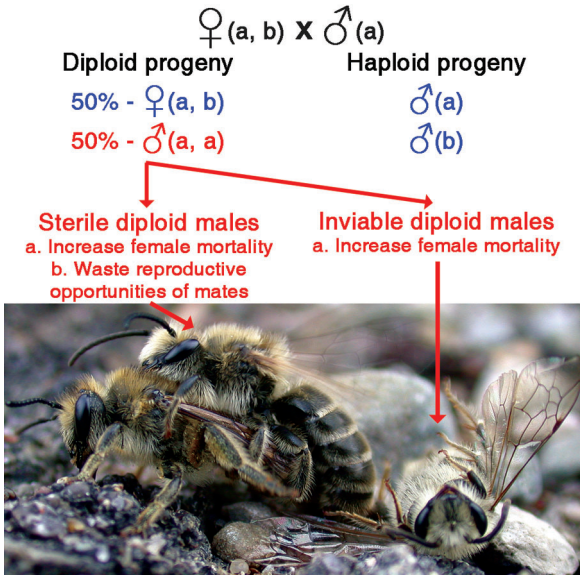

Fig. 1. The cost of sl-CSD. When haplodiploid females mate with males that share a *csd* allele in common (allele *a*), half of their diploid progeny will be homozygous at *csd* and will develop into DMs. Because females fertilize their eggs to produce daughters only, DMP is best viewed as increased female mortality. In some species, DMs have low viability. More often, however, DMs are effectively sterile: they are viable and achieve matings but do not father diploid daughters, thus reducing the reproductive success of their mates.

females mating with them produce inviable triploid daughters (refs. 17–19, but see ref. 34).

Each simulated generation proceeded as follows: (*i*) Females were assigned one mate at random, and males can remate. (*ii*) The base net reproductive output (X_e) for the generation is adjusted to account for environmental stochasticity by drawing a random number from a normal distribution with variance V_e. The number of offspring produced per female is then determined by randomly drawing numbers from a normal distribution with mean X_e and variance V_d, to account for demographic stochasticity. (*iii*) Offspring sex was determined stochastically by following a binomial distribution around the mean even sex ratio. In simulations with sl-CSD, offspring were randomly assigned parental sex alleles (diploids inherited an allele from each of their parents, whereas haploids inherited a maternal allele only). (*iv*) Any triploid daughters produced by females mated with DMs were killed. (*v*) If the number of progeny exceeded K, progeny were randomly killed until K was met (28). (*vi*) In simulations with sl-CSD, females homozygous at the sex locus were considered DMs. DMs were either killed when inviable or moved from the female matrix to the male matrix when effectively sterile. Steps *i–vi* comprise one generation. The simulation repeated steps *i–vi* until the 100th generation. The entire simulation was then iterated 1,000 times. The computer program used to conduct the stochastic simulations outlined above is available on request.

Extinction Risk. The probability of extinction [P(E)] was estimated as the proportion of iterations ending in extinction at the end of the simulation. Extinction occurred when both sexes had an abundance of zero. Relative extinction rate was estimated by multiplying P(E) by the inverse of average time to extinction. This equation is unsolvable when no iterations ended in extinction. For such cases, we assumed that the average time to extinction was so large that its inverse was ≈0, yielding a relative extinction rate of 0. A relative extinction rate of 100% was scaled to represent the maximum extinction rate experienced by any simulated population without DMP. Median time to extinction also can be used to quantify extinction risk; however, it must be estimated deterministically when simulated populations do not go extinct (11). We decided not to use this parameter to avoid unnecessary pooling of stochastic and deterministic data.

To examine how DMP compares with inbreeding depression-induced extinction risk in diploids, we compared our data with published estimates of P(E) for 16 threatened species (11), obtained by using a similar individual-based stochastic model, VORTEX (28). Estimates of P(E) from ref. 11 had been obtained by simulating diploid populations with and without inbreeding depression, modeled by assuming 3.14 lethal equivalents per diploid genome, including catastrophes, and were projected forward for an average of 26 generations given three population sizes (N = 50, 250, and 1,000; $K = 2N$). Because catastrophic events are not incorporated in our model, we reanalyzed the diploid data set by using VORTEX, given the input files supplied in ref. 11, excluding catastrophes. We then estimated the average increase in P(E) due to inbreeding depression by subtracting the P(E) of simulations with inbreeding depression from those without it. We conducted simulations of haplodiploid populations, with and without sl-CSD, initialized with the same demographic parameters (r and its variance), and projected for the same number of generations as each of the 16 diploid organisms surveyed, for the three population sizes outlined above. We estimated the increase in P(E) due to DMP by subtracting the P(E) of simulations with DMP from the P(E) of simulations without DMP. It is likely that inbreeding depression in diploids modeled by using 3.14 lethal equivalents, the median value found in a study of 40 captive vertebrate populations (35), represents an underestimate of that experienced in the wild (11). We investigated the robustness of our comparison by reanalyzing the diploid data set with inbreeding depression modeled by using twice the median value of lethal equivalents. This higher load resulted in an average increase of P(E) due to inbreeding depression from 9.9% to 15.1%, well below the increases caused by sl-CSD in haplodiploids, and did not change the outcome of our statistical analyses.

The number of *csd* alleles averaged over iterations from the population replicate with K = 500 and an average net reproductive output of two offspring per female were used to construct Fig. 3*B*. To illustrate differences in secondary sex ratio at extinction, the observed sex ratio at extinction in population replicates with K = 50 and an average net reproductive output of two offspring per female were used to construct Fig. 3*C*. These replicates were chosen because they provided the largest sample size for secondary sex ratio at extinction for populations with no sl-CSD.

Statistical Tests. We used the nonparametric Kruskal–Wallis test to compare the mean of P(E) and relative extinction rate between groups, including comparisons between haplodiploids with sl-CSD and diploids with inbreeding depression.

Results

We performed stochastic simulations of solitary isolated haplodiploid populations in the absence or presence of sl-CSD with inviable or effectively sterile DMs. The former model represents the base extinction risk due to demographic and environmental stochasticity alone, whereas models simulating DMP investigate its effects on extinction risk. DMP significantly increased the average probability of extinction, P(E), in our simulations (Fig. 2 *A–C*) by more than an order of magnitude with either inviable or effectively sterile males ($P < 0.0001$ for both models) over all simulated population sizes and reproductive rates. In the absence of DMP, demographic and environmental stochasticity caused extinction in mostly stable populations (two net offspring per female, $r = 0$) but not in replicates with higher reproductive rates

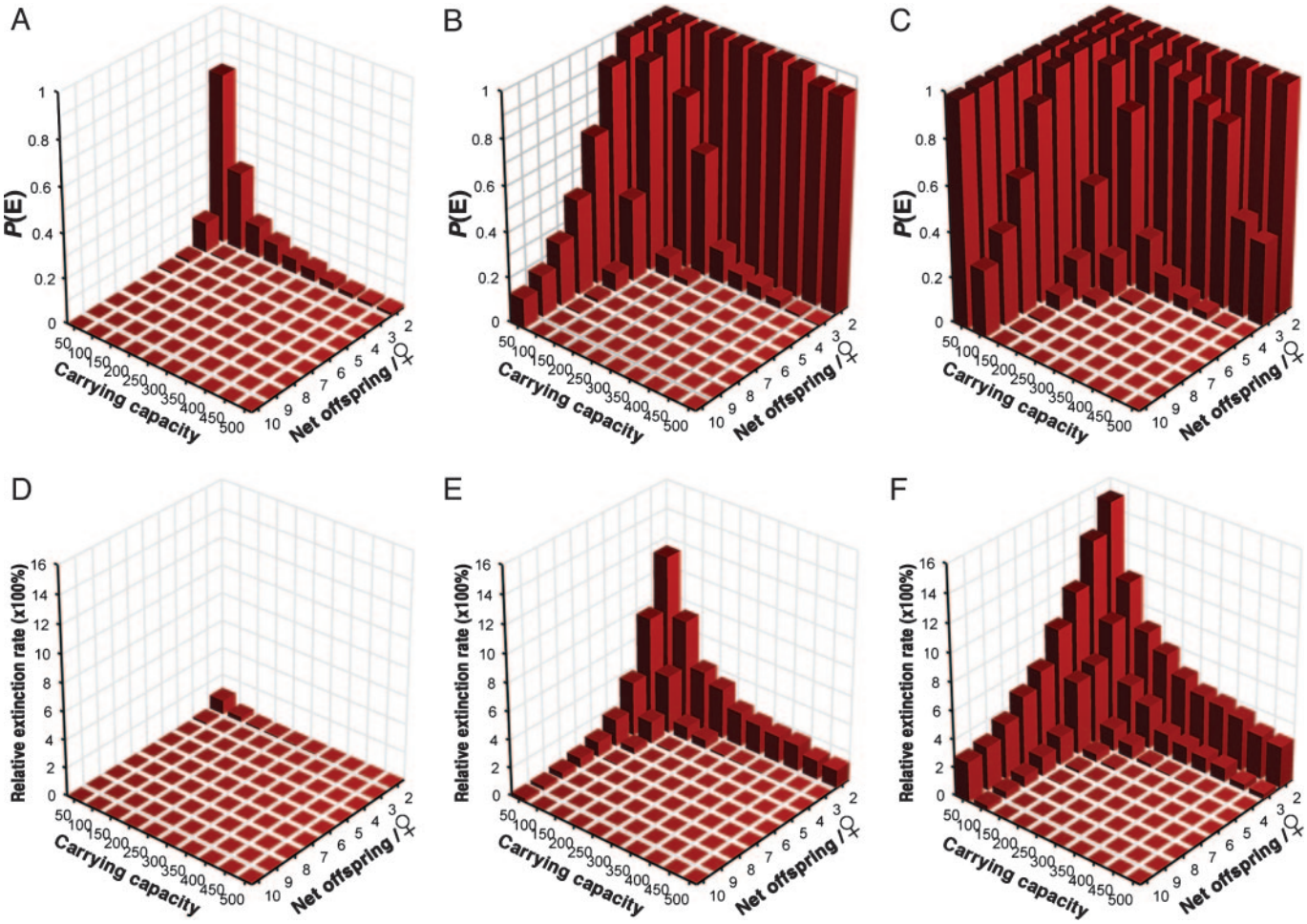

Fig. 2. Complementary sex determination elevates extinction risk. Compared with haplodiploid populations without sl-CSD (*A*), the production of DMs increases *P*(E) when DMs are inviable (*B*) or effectively sterile (*C*) over a range of population sizes and reproductive rates. Similarly, the relative extinction rate for haplodiploid populations without sl-CSD (*D*) is an order of magnitude lower on average than those of populations with inviable (*E*) and effectively sterile (*F*) DMs. Note that our analyses underestimate the actual extinction risk found in natural populations (see *Methods*).

(Fig. 2*A*). Conversely, *P*(E) was greatly elevated with DMP (Fig. 2 *B* and *C*), even in populations capable of comparatively high reproductive rates. DM sterility (Fig. 2*C*) was more detrimental than inviability (Fig. 2*B*). This difference is expected (25, 26), because in contrast with the production of inviable DMs, which contribute to increased female mortality over a single generation, the production of effectively sterile DMs actually increases effective female mortality over two generations, because females mating with sterile DMs produce triploid daughters. As *K* increased along with the effective number of *csd* alleles, *P*(E) decreased in populations capable of moderate and high growth rates but remained high for large populations with limited growth rates and DMP.

We found a dramatic decrease in the average time to extinction resulting from DMP with inviable or effectively sterile DMs. We estimated the relative extinction rate for all population replicates (Fig. 2 *D–F*). Relative extinction rates were at least an order of magnitude higher due to DMP with inviable (average relative extinct rate: 68.8%, $P < 0.0001$) or effectively sterile DMs (171.1%, $P < 0.0001$), compared with the relative base extinction rate (2.3%).

DMP, with either inviable or effectively sterile DMs, caused higher extinction probabilities than inbreeding depression in threatened diploid populations (data from ref. 11) when haplodiploid populations of sizes and demographic parameters similar to those of the diploids were modeled ($P < 0.05$ for all tests). The base *P*(E) in haplodiploid and diploid populations without DMP and inbreeding depression, respectively, did not differ significantly ($P = 0.69$). On average, DMP increased the base *P*(E) by 52.7% and 63.2% with inviable or effectively sterile males, respectively, whereas inbreeding depression increased *P*(E) by 9.9% in diploid populations. In simulations with medium population sizes ($N = 250$, $K = 500$), DMP increased *P*(E) in haplodiploids with inviable (69.2%) or effectively sterile (79.4%) DMs by over an order of magnitude above that caused by inbreeding depression in diploids (6.2%).

We call the increased extinction risk due to DMP in haplodiploids with sl-CSD the DM vortex, and it proceeds as follows (Fig. 3*A*). The production of DMs initially reduces population growth rates (25) and effective population sizes (26). In small closed populations, demographic and environmental stochasticity combined with increased drift further reduce the effective number of *csd* alleles (Fig. 3*B*), leading to higher levels of DMP. When each female on average fails to produce one daughter, negative population trends are inevitable, further exacerbating the effects of drift and demographic and environmental stochasticity. The cycle continues, ultimately to extinction. Populations in the DM vortex will experience a loss of females in successive generations, leading to highly male-biased secondary sex ratios at extinction (Fig.

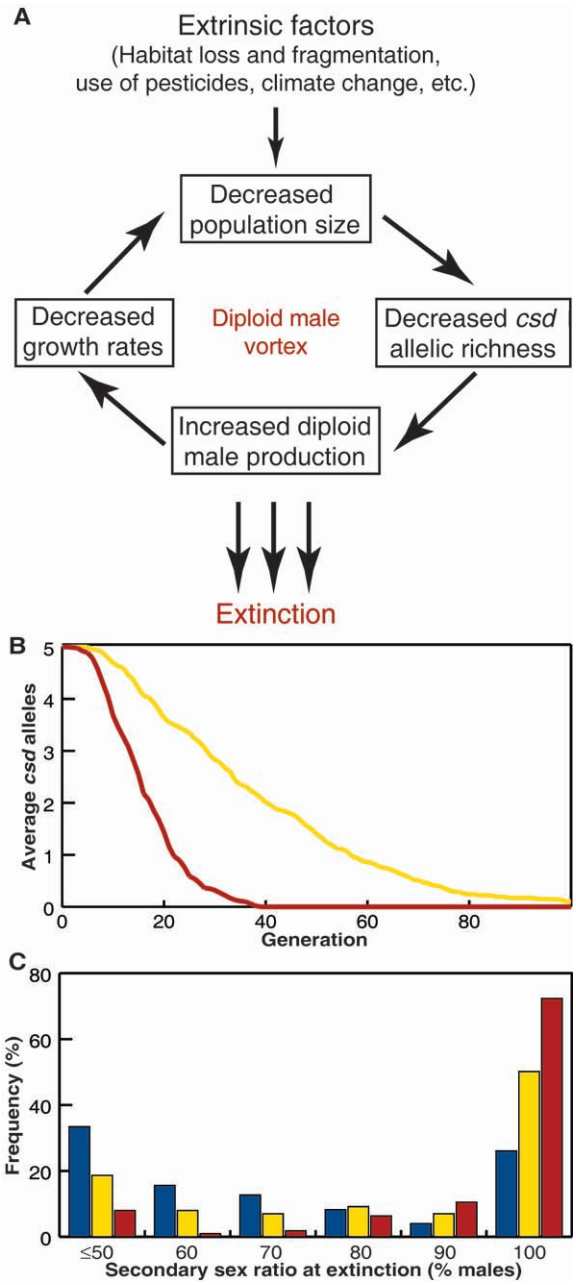

Fig. 3. The DM extinction vortex. (*A*) DMP in small isolated populations can initiate an extinction vortex. (*B*) The loss of *csd* alleles in successive generations leads to higher levels of DMP, given inviable (yellow line) or effectively sterile (red line) DMs. DMP acts to drain the population of females, causing reductions in population size. (*C*) The cycle ultimately results in extinction, with highly male-biased secondary sex ratios, consistent with observations of laboratory-inbred hymenopterans. Yellow and red bars represent populations with DMP with inviable or effectively sterile DMs, respectively, whereas blue bars represent populations without sl-CSD.

3*C*), which has been observed in laboratory hymenopteran populations (25, 36).

Discussion

As shown in Fig. 2, the role of DMP in causing extinction is extremely high in populations with low r and/or K values. Although there are exceptions (37), low fecundity is a common feature in solitary aculeate hymenopterans (29, 38–41), where females often produce a total of only 6–12 eggs in their lifetime. For example, females of the solitary specialist bee *Dieunomia triangulifera* produced a total of 2–6 eggs during their lifetime (29), and those of *Andrena erythronii* produced a total of 8 eggs (41). Similarly, in a survey based on 12 species of solitary nest-provisioning wasps, females produced an average of 9.8 eggs during their lifetime (39). Usually high preadult mortality rates (up to ≈50–60%; ref. 40), common entire nest failure (42), and potentially high adult predation rates (43) further combine to reduce net fecundities of solitary aculeate hymenopterans to levels where the DM vortex can be initiated. In addition, species that nest in preformed cavities (44) are expected to have low effective carrying capacities and contest competition for nest sites. Parasitoid wasps often have higher net fecundities than do solitary aculeate hymenopterans (39); however, high preadult mortality rates (45) result in low r values in some species.

Three other aspects of hymenopteran biology exacerbate extinction proneness through the DM vortex. First, a disproportionate number of species persist in highly viscous populations with low levels of gene flow, compared with other insects (5, 46). Second, many species have high levels of resource specialization (29, 39, 46–48). These aspects both serve to reduce effective population sizes and *csd* allelic richness (5, 48). Third, eusocial species with large colony sizes have particularly low effective population sizes (5). Although our simulations deal with solitary species only, DMP also is expected to reduce population viability for social species. A clear link between DMP and local extirpation exists for social bees (21) and ants (49).

CSD in haplodiploids is similar to self-incompatibility, which has been shown to reduce population viability in small plant populations (10). However, differences between the two systems suggest that sl-CSD poses a greater threat. First, empirical estimates of self-incompatibility alleles in plant populations (50) are larger than those of *csd* alleles in haplodiploid populations (15). Second, and of greater significance, self-incompatibility in plants is mostly prezygotic (10, 50), resulting in the loss of gametes, whereas sl-CSD in haplodiploids is postzygotic (15), resulting in the loss of progeny.

Although the role of genetic factors in extinction is well established for diploid organisms, our findings suggest that it is substantially more important for haplodiploids with sl-CSD. Despite its importance, genetic factors are generally ignored in haplodiploid conservation biology. For example, genetic aspects of the worldwide decline of pollinators are not addressed in *International Pollinators Initiative: The São Paulo Declaration on Pollinators* (6). Our results indicate that even large populations with limited growth rates can be susceptible to extinction (Fig. 2). If declines in pollination services (3) can be taken as evidence for negative growth rates in pollinator populations, then many bees may have already been committed to extinction through the DM vortex. Given the large impact of DMP on extinction risk, we strongly recommend that haplodiploid populations targeted for conservation be managed to reduce the genetic load associated with DMP. The elevated extinction proneness of the haplodiploid Hymenoptera, combined with the keystone services that they provide, should make them excellent indicators for assessing the health of natural and agricultural ecosystems (2, 24). This elevated extinction risk results from the sex-determining mechanism that essentially makes all sex alleles lethal when homozygous.

We thank J. C. Grixti, S. I. Wright, J. Shore, and two anonymous reviewers for helpful comments on the manuscript. This work was supported by grants from the Natural Sciences and Engineering Research Council (Canada) (to L.P.) and a Canada Graduate Scholarship (to A.Z.).

1. Wilson, E. O. (1987) *Conserv. Biol.* **1,** 344–346.
2. LaSalle, J. & Gauld, I. D. (1993) *Hymenoptera and Biodiversity* (C.A.B. International, Oxon, U.K.).
3. Kevan, P. G. & Viana, B. F. (2004) *Biodiversity* **4,** 3–8.
4. Allen-Wardell, G., Bernhardt, P., Bitner, R., Burquez, A., Buchmann, S. L., Cane, J. H., Cox, P. A., Dalton, V., Feinsinger, P., Ingram, M., *et al*. (1998) *Conserv. Biol.* **12,** 8–17.
5. Packer, L. & Owen, R. (2001) *Conserv. Ecol.* **5,** 4, www.consecol.org/vol5/iss1/art4.
6. Dias, B. S. F., Raw, A. & Imperatri-Fonseca, V. L. (1999) *International Pollinators Initiative: The São Paulo Declaration on Pollinators* (Brazilian Ministry of the Environment, Brasilia). Available at www.biodiv.org/doc/case-studies/agr/cs-agr-pollinator-rpt.pdf.
7. Matheson, A., Buchmann, S. L., O'Toole, C., Westrich, P. & Williams, I. (1996) *The Conservation of Bees* (Academic, London).
8. Spielman, D., Brook, B. W. & Frankham, R. (2004) *Proc. Natl. Acad. Sci. USA* **101,** 15261–15264.
9. Saccheri, I., Kuussaari, M., Kankare, M., Vikman, P., Fortelius, W. & Hanski, I. (1998) *Nature* **392,** 491–494.
10. Frankham, R., Ballou, J. D. & Briscoe, D. A. (2002) *Introduction to Conservation Genetics* (Cambridge Univ. Press, Cambridge, U.K.).
11. Brook, B. W., Tonkyn, D. W., O'Grady, J. J. & Frankham, R. (2002) *Conserv. Ecol.* **6,** 16, www.consecol.org/vol6/iss1/art16.
12. Hedrick, P. W. & Parker, J. D. (1997) *Annu. Rev. Ecol. Syst.* **28,** 55–83.
13. Werren, J. H. (1993) in *The Natural History of Inbreeding and Outbreeding: Theoretical and Empirical Perspectives*, ed. Thornhill, N. W. (Univ. of Chicago Press, Chicago), pp. 42–59.
14. Luna, M. G. & Hawkins, B. A. (2004) *Environ. Entomol.* **33,** 765–775.
15. Cook, J. M. & Crozier, R. H. (1995) *Trends Ecol. Evol.* **10,** 281–286.
16. Beye, M., Hasselmann, M., Fondrk, M. K., Page, R. E. & Omholt, S. W. (2003) *Cell* **114,** 419–429.
17. Krieger, M. J. B., Ross, K. G., Chang, C. W. & Keller, L. (1999) *Heredity* **82,** 142–150.
18. Liebert, A. E., Johnson, R. N., Switz, G. T. & Starks, P. T. (2004) *Insect Soc.* **51,** 205–211.
19. Ayabe, T., Hoshiba, H. & Ono, M. (2004) *Chromosome Res.* **12,** 215–223.
20. Butcher, R. D. J., Whitfield, W. G. F. & Hubbard, S. F. (2000) *J. Evol. Biol.* **13,** 593–606.
21. Carvalho, G. A. (2001) *J. Hymn. Res.* **10,** 10–15.
22. Heimpel, G. E., Antolin, M. F. & Strand, M. R. (1999) *Heredity* **82,** 282–291.
23. Zayed, A. & Packer, L. (2001) *Heredity* **87,** 631–636.
24. Zayed, A., Roubik, D. W. & Packer, L. (2004) *Proc. R. Soc. Lond. Ser. B* **271,** S9–S12.
25. Stouthamer, R., Luck, R. F. & Werren, J. H. (1992) *Environ. Entomol.* **21,** 427–435.
26. Zayed, A. (2004) *Heredity* **93,** 627–630.
27. Gabriel, W. & Ferrière, R. (2004) in *Evolutionary Conservation Biology*, eds. Ferrière, R., Dieckmann, U. & Couvet, D. (Cambridge Univ. Press, Cambridge, U.K.), pp. 19–40.
28. Lacy, R. C. (1993) *Wildl. Res.* **20,** 45–64.
29. Minckley, R. L., Wcislo, W. T., Yanega, D. & Buchmann, S. L. (1994) *Ecology* **75,** 1406–1419.
30. Tepedino, V. J. & Torchio, P. F. (1982) *Ecol. Entomol.* **7,** 453–462.
31. Kerr, W. E. (1997) *Braz. J. Genet.* **20**(4).
32. Cornuet, J. M. (1980) *J. Apic. Res.* **19,** 3–5.
33. Eickwort, G. C. & Ginsberg, H. S. (1980) *Annu. Rev. Entomol.* **25,** 421–446.
34. Cowan, D. P. & Stahlhut, J. K. (2004) *Proc. Natl. Acad. Sci. USA* **101,** 10374–10379.
35. Ralls, K., Ballou, J. D. & Templeton, A. (1988) *Conserv. Biol.* **2,** 185–193.
36. Wu, Z., Hopper, K. R., Ode, P. J., Fuester, R. W., Chen, J. & Heimpel, G. E. (2003) *Entomol. Sin.* **10,** 81–93.
37. Danforth, B. N. (1990) *Behav. Ecol. Sociobiol* **27,** 159–168.
38. Else, G., Felton, J. & Stubbs, A. (1978) *The Conservation of Bees and Wasps* (Nature Conservancy Council, Peterborough, U.K.).
39. O'Neil, K. M. (2000) *Solitary Wasps: Behavior and Natural History* (Comstock, Ithaca, NY).
40. Danks, H. V. (1971) *J. Anim. Ecol.* **40,** 79–82.
41. Michener, C. D. & Rettenmeyer, C. W. (1956) *Univ. Kans. Sci. Bull.* **37,** 645–684.
42. Richards, M. H. & Packer, L. (1995) *Can. J. Zool.* **73,** 933–941.
43. Dukas, R. (2001) in *Cognitive Ecology of Pollination*, eds. Chittka, L. & Thomson, J. (Cambridge Univ. Press, Cambridge, U.K.), pp. 214–236.
44. Krombein, K. V. (1967) *Trap-Nesting Wasps and Bees: Life Histories, Nests, and Associates* (Smithsonian Press, Washington, DC).
45. Nagelkerke, C. J. & Hardy, I. C. W. (1994) *Behav. Ecol.* **5,** 401–411.
46. Danforth, B. N., Ji, S. & Ballard, L. J. (2003) *J. Kans. Entomol. Soc.* **76,** 221–235.
47. Michener, C. D. (2000) *The Bees of the World* (Johns Hopkins Univ. Press, Baltimore).
48. Packer, L., Zayed, A., Grixti, J. C., Ruz, L., Owen, R. E., Vivallo, F. & Toro, H. (2005) *Conserv. Biol.* **19,** 195–202.
49. Ross, K. G. & Fletcher, D. J. C. (1986) *Behav. Ecol. Sociobiol.* **19,** 283–291.
50. Lawrence, M. J. (2000) *Ann. Bot. (London)* **85,** 221–226.