

Science

 AAAS

**Conditional Use of Sex and Parthenogenesis for
Worker and Queen Production in Ants**

Morgan Pearcy, *et al.*
Science **306**, 1780 (2004);
DOI: 10.1126/science.1105453

***The following resources related to this article are available online at
www.sciencemag.org (this information is current as of December 15, 2008):***

Updated information and services, including high-resolution figures, can be found in the online version of this article at:

<http://www.sciencemag.org/cgi/content/full/306/5702/1780>

Supporting Online Material can be found at:

<http://www.sciencemag.org/cgi/content/full/306/5702/1780/DC1>

A list of selected additional articles on the Science Web sites **related to this article** can be found at:

<http://www.sciencemag.org/cgi/content/full/306/5702/1780#related-content>

This article **cites 16 articles**, 4 of which can be accessed for free:

<http://www.sciencemag.org/cgi/content/full/306/5702/1780#otherarticles>

This article has been **cited by** 31 article(s) on the ISI Web of Science.

This article has been **cited by** 5 articles hosted by HighWire Press; see:

<http://www.sciencemag.org/cgi/content/full/306/5702/1780#otherarticles>

This article appears in the following **subject collections**:

Evolution

<http://www.sciencemag.org/cgi/collection/evolution>

Information about obtaining **reprints** of this article or about obtaining **permission to reproduce this article** in whole or in part can be found at:

<http://www.sciencemag.org/about/permissions.dtl>

Many variations of the method are possible. The activity and affect descriptors used to describe episodes should fit the particular topic of investigation. Interactive questionnaires offer further opportunities to tailor the affect terms to the respective episode; for example, when the individual identifies an episode as an interaction with customers rather than with family members, different descriptors could be presented. Other variations could make the method more practical for adoption in conventional surveys. Our preliminary work suggests that much of the benefit of the DRM in producing accurate emotional recall could be retained if respondents are asked to retrieve specific recent episodes of a designated type (e.g., "the last occasion on which you went out to dinner"). When samples are large and interviewing time is scarce, the allocation of different situations to subgroups of respondents makes it possible to achieve comprehensive coverage of situations while minimizing respondent burden. In conjunction with time-use data obtained from other sources, affect profiles of the main activities in which people engage could be integrated to produce a duration-weighted assessment of the experience of the population and of subgroups. The DRM or its variants could also contribute to the development of an accounting system for the well-being of society, a potentially important tool for social policy (32, 33).

References and Notes

1. M. Bergner, M. L. Rothman, *Annu. Rev. Public Health* **8**, 191 (1987).
2. S. Cohen, W. Doyle, R. Turner, C. Alper, D. Skoner, *Psychol. Sci.* **14**, 389 (2003).
3. M. Marmot, *The Status Syndrome* (Bloomsbury, London, 2004).
4. F. T. Juster, in *Time, Goods, and Well-Being*, F. Juster, F. Stafford, Eds. (Institute for Social Research, Ann Arbor, MI, 1985), pp. 397–414.
5. P. van de Ven, B. Kazemier, S. Keuning, *Measuring Well-Being with an Integrated System of Economic and Social Accounts* (Department of National Accounts, Voorburg, Netherlands, 1999).
6. F. Y. Edgeworth, *Mathematical Psychics: An Essay on the Application of Mathematics to the Moral Sciences* (C. K. Paul, London, 1881).
7. D. Kahneman, P. Wakker, R. Sarin, *Q. J. Econ.* **112**, 375 (1997).
8. G. Dow, J. F. Juster, in *Time, Goods, and Well-Being*, F. Juster, F. Stafford, Eds. (Institute for Social Research, Ann Arbor, 1985), pp. 397–413.
9. L. Flood, *Household, Market, and Nonmarket Activities: Procedures and Codes for the 1993 Time-Use Survey* (Uppsala Univ. Dept. Economics, Uppsala, Sweden, 1997), vol. VI.
10. A. Campbell, *The Sense of Well-Being in America* (McGraw-Hill, New York, 1981).
11. F. M. Andrews, S. B. Whithey, *Social Indicators of Well-Being: Americans' Perceptions of Life Quality* (Plenum, New York, 1976).
12. M. Csikszentmihalyi, R. E. Larsen, *J. Nerv. Ment. Dis.* **175**, 526 (1987).
13. A. A. Stone, S. S. Shiffman, M. W. DeVries, in *Well-Being: The Foundations of Hedonic Psychology*, D. Kahneman, E. Diener, N. Schwarz, Eds. (Russell-Sage, New York, 1999), pp. 61–84.
14. M. D. Robinson, G. L. Clore, *Psychol. Bull.* **128**, 934 (2002).
15. R. Belli, *Memory* **6**, 383 (1998).

16. Materials and methods are available as supporting material on *Science Online*.
17. A. A. Stone et al., *J. Pers. Soc. Psychol.* **74**, 1670 (1998). Respondents with high levels of job stress were recruited in New York ($n = 88$ men, 91 women; average age 40.0 years; 89% white; 58% married; 50% had some college). Situations and affect descriptors were similar to the DRM study.
18. K. N. Fortson, *Monthly Labor Rev.* **127**, 18 (2004).
19. We conducted an auxiliary random-digit-dial phone survey of 69 women, who ranked the activities from most to least enjoyable. Kendall's W coefficient of concordance among the ranks was 0.47, slightly higher than the concordance of economists' rankings of journals (20).
20. K. Axarloglou, V. Theoharakis, *J. Euro. Econ. Assoc.* **1**, 1402 (2003).
21. Thirty-nine job features (e.g., tenure and job level) were entered into a regression to predict duration-weighted enjoyment at work (16).
22. N. K. Semmer, S. Grebner, A. Elfering, in *Research in Occupational Stress and Well-being*, P. Perrewé, D. Ganster, Eds. (JAI, Amsterdam, 2004), vol. 3, pp. 207–263.
23. Twenty personal features (e.g., age and marital status) predicted duration-weighted enjoyment at work (16).
24. We computed enjoyment at home for respondents who reported "no problem at all" in keeping up "enough enthusiasm to get things done" during the last month and for respondents who reported "somewhat of a problem." The difference between the groups spanned 46% of the enjoyment scale.

25. R. A. Easterlin, *J. Econ. Behav. Org.* **27**, 35 (1995).
26. P. Brickman, D. T. Campbell, in *Adaptation-Level Theory*, M. Appley, Ed. (Academic Press, New York, 1971), pp. 215–231.
27. The first question in the survey was "How satisfied are you with your life as a whole these days? Are you very satisfied, satisfied, not very satisfied, or not at all satisfied?".
28. D. Kahneman, in *Choices, Values and Frames*, D. Kahneman, A. Tversky, Eds. (Cambridge Univ. Press, New York, 2000), chap. 38.
29. D. A. Schkade, D. Kahneman, *Psychol. Sci.* **9**, 340 (1998).
30. S. Lyubomirsky, K. M. Sheldon, D. Schkade, *Rev. Gen. Psychol.*, in press.
31. G. Becker, *Econ. J.* **75**, 493 (1965).
32. D. Kahneman, A. B. Krueger, D. A. Schkade, N. Schwarz, A. A. Stone, *Am. Econ. Rev.* **94**, 429 (2004).
33. E. Diener, M. Seligman, *Psychol. Sci. Pub. Int.* **5**, 1 (2004).
34. This research was supported by the William and Flora Hewlett Foundation, and also by the Woodrow Wilson School of Public and International Affairs at Princeton, the NSF, and the National Institute on Aging. Order of authorship is alphabetical. D.K. is affiliated with the Gallup Organization. A.A.S. holds equity in and is the Associate Chair of the Scientific Advisory Board of invivodata, Inc., a company that markets an electronic patient diary system.

Supporting Online Material

www.sciencemag.org/cgi/content/full/306/5702/1776/DC1
Materials and Methods

2 August 2004; accepted 30 September 2004

Conditional Use of Sex and Parthenogenesis for Worker and Queen Production in Ants

Morgan Pearcy,¹ Serge Aron,^{1*} Claudie Doums,² Laurent Keller³

The near-ubiquity of sexual reproduction in animal species has long been considered a paradox because sexually reproducing individuals transmit only half of their genome to their progeny. Here, we show that the ant *Cataglyphis cursor* circumvents this cost by using alternative modes of reproduction for the production of reproductive and nonreproductive offspring. New queens are almost exclusively produced by parthenogenesis, whereas workers are produced by normal sexual reproduction. By selectively using sex for somatic growth and parthenogenesis for germline production, *C. cursor* has taken advantage of the ant caste system to benefit from the advantages of both sexual and asexual reproduction.

The main advantage of asexual reproduction is that it confers a twofold advantage over sexuality by allowing, generation by generation, the transmission of twice the number of genes to offspring (1, 2). However, asexual reproduction is also associated with both short-term and long-term disadvantages, including a lower genetic diversity of offspring

and a reduced rate of adaptive evolution of species (3, 4). The nature and the degree of the cost associated with asexual reproduction is expected to vary across taxa, depending on the biology of the species and the type of environment in which they live (1–3).

In ants, as in other Hymenoptera, females are usually produced by sexual reproduction and are diploid, whereas males develop from unfertilized eggs and are haploid (3). The diploid fertilized eggs can develop into either new queens (gynes) or workers, with the developmental switch generally under environmental control (5). In the Cape honey bee and five ant species, however, unmated workers may reproduce by thelytokous parthenogenesis (6–11); that is, they may produce female

¹Behavioural and Evolutionary Ecology, CP 160/12, Université Libre de Bruxelles, av. F. D. Roosevelt 50, B-1050 Brussels, Belgium. ²Fonctionnement et Evolution des Systèmes Ecologiques, Université Paris VI, 7 quai St. Bernard, 75252 Paris Cedex 05, France. ³Department of Ecology and Evolution, Bâtiment de Biologie, CH-1015 Lausanne, Switzerland.

*To whom correspondence should be addressed. E-mail: saron@ulb.ac.be

offspring from unfertilized eggs. While conducting a population genetic study of one of these species, the ant *Cataglyphis cursor*, we discovered that not only unmated workers but also mated queens can use thelytokous parthenogenesis. Pedigree analyses indicated that queens use automictic parthenogenesis with central fusion where two of the four products of meiosis merge. Unlike workers, queens use this mode of reproduction specifically to produce new queens.

Cataglyphis cursor is a common ant in the dry forests of Europe. Colonies usually contain a single queen and up to 3000 workers. Only few colonies produce gynes, and the number of gynes produced per colony is small. This is because *C. cursor* has an unusual mating system whereby gynes mate near the parental nest before leaving the colony with adult workers to initiate new colonies 3.2 to 11.3 m away (12). Previous studies also indicate that *C. cursor* workers can produce both gynes and workers parthenogenetically in colonies that have lost the mother queen (7).

We collected 38 large colonies in Southern France and genotyped 532 workers at four highly polymorphic microsatellite loci (expected heterozygosities, 0.833 to 0.944) (13). The genotypes indicated that 35 of these colonies contained a single reproductive queen (monogyny), whereas three colonies contained offspring from at least two queens. Analysis of lab-raised worker progeny ($n = 437$ freshly eclosed workers) from 12 queens showed that they had mated with an average of 5.6 ± 1.3 males (range, 4 to 8).

A detailed analysis of the 35 monogynous colonies showed that most of the workers in these colonies could only have been produced by sexual reproduction. Overall, 476 of the 489 workers (97.3%) genotyped in the 35 colonies harbored, at one or several loci, alleles that were not present in the mother queen and came from one of the queen's mates. It is impossible to determine whether the 13 workers harboring only alleles identical to those of their mother were fathered by a male that had no allele distinct from those of the queen or whether they had been parthenogenetically produced. Because the four microsatellite loci were highly polymorphic, the probability of mating with a male harboring no diagnostic allele at any of

the four loci was low, ranging from 0.0001 to 0.013 across colonies according to the queen's genotype. Thus, of the 476 workers, only one was expected to have no diagnostic alleles. Hence, it is likely that some or all of the 13 workers with no diagnostic paternal allele may indeed have been asexually produced (the estimated proportion of asexually produced workers is 2.5% when corrected for the probability of nondetection of paternal alleles).

A total of 56 gynes were produced by 10 of the 35 monogynous colonies. In contrast to workers, most of these gynes (54 of 56) had alleles at the four loci that could all be attributed to the queen (Fig. 1A), hence these gynes had been produced by parthenogenesis. The alternative explanation, that these 54 gynes had been fathered by a male having no diagnostic alleles, can be ruled out. Queens and males came from the same gene pool, as indicated by a lack of significant difference in allele frequencies for the four loci (Fisher exact test, all $P > 0.05$) and the workers' *Fis* value (an index of observed versus expected homozygosity), which was not significantly different from zero ($Fis = 0.011 \pm 0.015$, $n = 35$ colonies; two-tailed t test, $t = 0.691$, $P = 0.494$). This, together with the high allelic diversity, resulted in a very high probability to detect a male's genetic contribution. However, none of the gynes produced in nine of the 10 colonies had any diagnostic allele, even though the likelihood of such a matched mating was lower than 0.013 in each of the nine colonies (range, 0.0001 to 0.013). Overall, the probability that all the fathers of the gynes produced in the nine

colonies had no diagnostic alleles was $P < 10^{-28}$. Indeed, the genotypes of workers in these nine colonies confirmed that all or most of the males that mated with the queens had diagnostic alleles at one or more loci (Fig. 1B). The outcome of the vast majority of gynes being produced by parthenogenesis was that the relatedness between queens and gynes was very high ($r = 0.864 \pm 0.046$, $n = 56$ gynes) and significantly greater ($P < 0.001$) than the theoretical value of 0.50 expected under sexual reproduction.

Most of the 54 parthenogenetic gynes were neither genetically identical to each other within a colony nor genetically identical to their mother queen. The discrepancies resulted from gynes being homozygous at some loci where the mother queen was heterozygous. In all cases, the gynes were homozygous for one of the two maternal alleles. This is the expected pattern under automictic parthenogenesis with central fusion. Because two of the four products of meiosis merge, the offspring have the same genotype as their mother for the loci that did not cross over, whereas the offspring is homozygous for one of the two maternal alleles if crossing-over did occur (14, 15). The frequency of transition from heterozygosity is expected to vary across loci depending on their distance to the centromere (15). Consistent with this prediction, the frequency of transition from heterozygosity to homozygosity varied significantly across the four loci, presumably reflecting differences in the distance between each locus and the centromere (Table 1; $\chi^2 = 25.53$, $P < 0.0001$).

The expected outcome of automictic parthenogenesis is a gradual increase in homo-

Table 1. Proportion of gynes homozygous for a given locus when the mother was heterozygous at that locus. The sample size for each locus is indicated.

Locus	Sample size	Percentage of gynes homozygous
<i>Ccur11</i>	53	5.7
<i>Ccur46</i>	47	46.8
<i>Ccur58</i>	47	34.0
<i>Ccur63b</i>	41	17.1

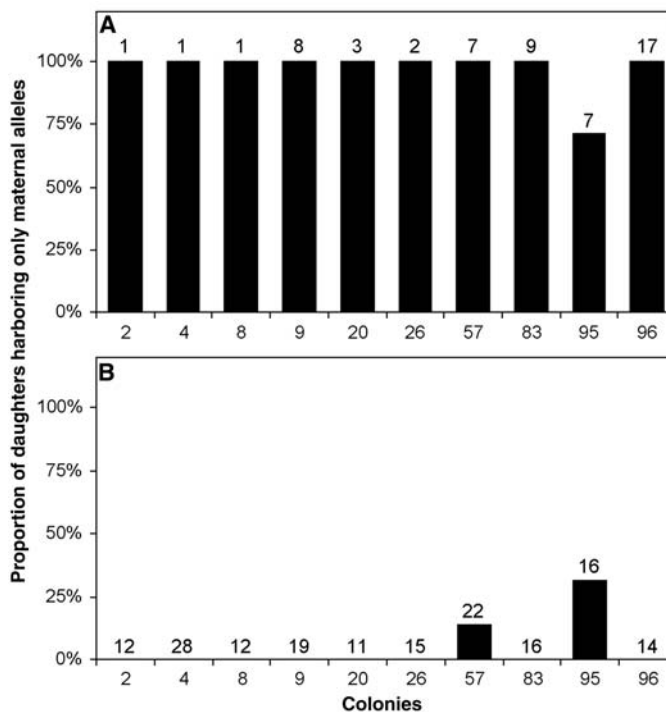


Fig. 1. Respective proportion of gynes (A) and workers (B) harboring maternal alleles only, and therefore interpreted as parthenogenetic daughters, in each of 10 colonies (colony numbers are laboratory designations). The sample size for each colony is indicated above the bars.

zygosity over time (16). Indeed, the overall level of homozygosity was significantly higher in gynes than in workers (Fig. 2; Fisher's exact test on the number of homozygous versus heterozygous loci in gynes and workers: $P < 0.0001$). Accordingly, F statistics revealed a significant excess of homozygosity in gynes ($F = 0.396 \pm 0.12$, $P < 0.001$) and queens ($F = 0.255 \pm 0.051$, $P < 0.001$) but not in workers from the same colonies ($F = 0.002 \pm 0.016$, $P = 0.45$). By increasing the levels of homozygosity, parthenogenesis should result in reduced queen survival and fitness, much like inbreeding does. However, the fitness effect might be limited for ant queens because they stay in the protected environment of the nest, except during colony founding. Even at this stage, the intensity of this cost should vary according to the mode of colony founding, with selection against more homozygous queens being higher in species where queens start a new colony on their own and lower in species, such as *C. cursor*, where queens do not go through a stage of independent colony founding (12).

In addition, two processes appear to counteract the process of genetic homogenization induced by automictic parthenogenesis. The first is the occasional production of gynes by sexual reproduction. The overall production of such gynes was 3.6% (2 of 56) in the 10 colonies studied. The second process is the occasional queen production by worker parthenogenesis. Because workers are usually produced by sexual reproduction, their contribution to gyne production will contribute to the maintenance of heterozygosity in gynes and queens, just as under queen sexual reproduction.

Although *C. cursor* queens do not require mating to produce diploid offspring, they have retained sexual reproduction to produce workers, which suggests that sexual reproduction has important benefits for colony function. The observed mating frequencies in

this species lie on the high end of the continuum of mating frequencies reported in ants (17). A possible explanation is that genetic input from an increased number of mates compensates for the negative effect of high queen homozygosity on colony genetic diversity. Parthenogenetic production of workers at the level observed for gynes would lower colony genetic diversity, which could lead to reduced defense against parasites, less efficient division of labor, and a decreased range of environmental conditions that a colony can tolerate (18–20). These costs are akin to those thought to lead to the instability of parthenogenetic reproduction in nonsocial organisms (2). Multiple mating lowered the overall relatedness of nestmate workers to $r = 0.42$ ($SE_{\text{jackknife}} = 0.02$, $n = 35$), a value well within the range of values reported in other ants (21). Thus, the high queen mating frequency may cancel out reduced genetic diversity at the colony level stemming from the relatively high queen homozygosity.

Using alternative modes of reproduction for the queen and worker castes may also enhance cooperation within the social group by aligning the interests of queens and workers. Parthenogenetic production of gynes by queens reduces conflict with workers because, just like queens, workers are significantly more closely related ($t = 2.31$, $df = 43$, $P = 0.03$) to the parthenogenetic gynes ($r = 0.59$, $SE_{\text{jackknife}} = 0.07$, $n = 10$) than they would be to sexually produced gynes or to gynes produced parthenogenetically by other workers (these two values are identical to the relatedness between workers, $r = 0.42$). As a result, workers should police the reproduction of other workers (22). The almost complete lack of worker-produced gynes in colonies containing a queen is consistent with this idea.

Conditional use of parthenogenesis for queen production might also occur in other ants, yet it may remain unnoticed because it

primarily occurs in dependent-founding species where it is most difficult to detect. In ants there is a strong association between the mode of colony founding and the number of queens, with dependent colony founding being almost exclusively restricted to species with high numbers of queens per nest (23, 24). The likelihood of detecting parthenogenesis with genetic markers is low in such species because it is very difficult to determine the maternity of female offspring. As a result, only a handful of studies in highly polygynous ants are sufficiently detailed to have enabled the detection of parthenogenesis.

This study shows that by taking advantage of the social caste system, *C. cursor* colonies can benefit from the advantages of both sexual and asexual reproduction. By using alternative modes of reproduction for the queen and worker castes, queens can increase the transmission rate of their genes to their reproductive female offspring while maintaining genetic diversity and social cohesion in the worker population. These findings, together with those of other recent genetic studies (25–29), indicate greater flexibility of the ant reproductive and social systems, thus providing an ideal ground to test various evolutionary predictions.

References and Notes

1. G. C. Williams, *Sex and Evolution* (Princeton Univ. Press, Princeton, NJ, 1975).
2. J. Maynard Smith, *The Evolution of Sex* (Cambridge Univ. Press, Cambridge, 1978).
3. G. Bell, *The Masterpiece of Nature: The Evolution and Genetics of Sexuality* (Univ. of California Press, Berkeley, CA, 1982).
4. S. A. West, C. M. Lively, A. F. Read, *J. Evol. Biol.* **12**, 1003 (1999).
5. E. O. Wilson, *The Insect Societies* (Harvard Univ. Press, Cambridge, MA, 1971).
6. S. Verma, F. Ruttner, *Apidologie (Celle)* **14**, 41 (1983).
7. H. Cagniant, *Insect. Soc.* **26**, 51 (1979).
8. T. Itow et al., *Insect. Soc.* **31**, 87 (1984).
9. K. Tsuji, K. Yamauchi, *Insect. Soc.* **42**, 333 (1995).
10. J. B. Heinze, B. Hölldobler, *Naturwissenschaften* **82**, 40 (1995).
11. D. A. T. Grasso, T. Wenseleers, A. Mori, F. Le Moli, J. Billen, *Ethol. Ecol. Evol.* **12**, 309 (2000).
12. A. Lenoir, L. Quérard, N. Pondicq, F. Bertoin, *Psyche (Stuttgart)* **95**, 21 (1988).
13. See supporting data on Science Online.
14. E. Suomalainen, A. Saura, J. Lokki, *Cytology and Evolution in Parthenogenesis* (CRC Press, Boca Raton, FL, 1987).
15. E. Baudry et al., *Genetics*, in press.
16. J.-C. Simon, F. Delmotte, C. Rispe, T. Crease, *Biol. J. Linn. Soc.* **79**, 151 (2003).
17. J. E. Strassmann, *Insect. Soc.* **48**, 1 (2001).
18. P. W. Sherman, T. D. Seeley, H. K. Reeve, *Am. Nat.* **131**, 602 (1988).
19. R. H. Crozier, R. E. Page, *Behav. Ecol. Sociobiol.* **18**, 105 (1985).
20. B. J. Cole, D. C. Wiernasz, *Science* **285**, 891 (1999).
21. R. H. Crozier, P. Pamilo, *Evolution of Social Insect Colonies: Sex Allocation and Kin Selection* (Oxford Univ. Press, Oxford, 1996).
22. F. L. W. Ratnieks, *Am. Nat.* **132**, 217 (1988).
23. L. Keller, *Ethol. Ecol. Evol.* **3**, 307 (1991).
24. A. F. G. Bourke, N. R. Franks, *Social Evolution in Ants* (Princeton Univ. Press, Princeton, NJ, 1995).
25. S. Helms Cahan et al., *Proc. R. Soc. London Ser. B.* **269**, 1871 (2002).

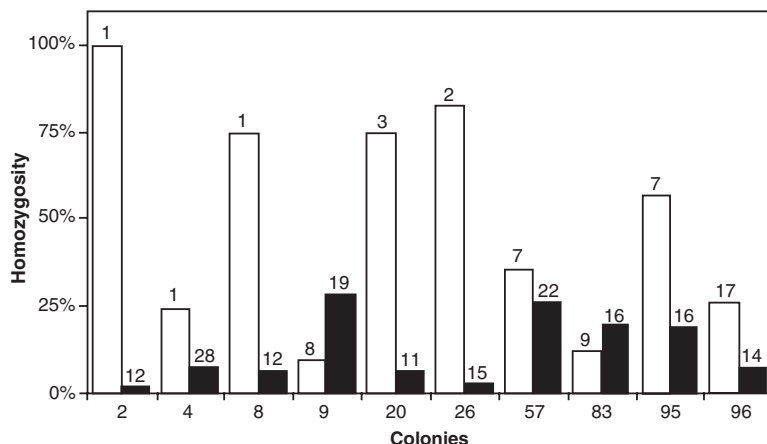


Fig. 2. Overall homozygosity detected in gynes (white) and workers (black) at all four loci, for each of 10 colonies. The sample size for each colony is indicated above the bars.

26. G. E. Julian, J. H. Fewell, J. Gadau, R. A. Johnson, D. Larrabee, *Proc. Natl. Acad. Sci. U.S.A.* **99**, 8157 (2002).
 27. V. P. Volny, D. M. Gordon, *Proc. Natl. Acad. Sci. U.S.A.* **99**, 6108 (2002).
 28. S. Helms Cahan, L. Keller, *Nature* **424**, 306 (2003).
 29. A. Hartmann, J. Wantia, J. A. Torres, J. Heinze, *Proc. Natl. Acad. Sci. U.S.A.* **100**, 12836 (2003).
 30. We thank L. Beukeboom, M. Chapuisat, P. Christe,

R. Hammond, S. H. Cahan, J. M. Pasteels, K. Parker, and I. Sanders for their comments on the manuscript. Supported by a doctoral grant from Fonds pour la Recherche dans l'Industrie et l'Agriculture (M.P.) and grants from the Belgian Fond National de la Recherche Scientifique (S.A.), the French Ministry of Research "Action concertée incitative jeunes chercheurs 2001" (C.D.), and the Swiss National Science Foundation (L.K.).

Supporting Online Material
www.sciencemag.org/cgi/content/full/306/5702/1780/DC1
 Materials and Methods
 References

21 September 2004; accepted 5 October 2004

Status and Trends of Amphibian Declines and Extinctions Worldwide

Simon N. Stuart,^{1*} Janice S. Chanson,¹ Neil A. Cox,¹
 Bruce E. Young,² Ana S. L. Rodrigues,³ Debra L. Fischman,³
 Robert W. Waller³

The first global assessment of amphibians provides new context for the well-publicized phenomenon of amphibian declines. Amphibians are more threatened and are declining more rapidly than either birds or mammals. Although many declines are due to habitat loss and overutilization, other, unidentified processes threaten 48% of rapidly declining species and are driving species most quickly to extinction. Declines are nonrandom in terms of species' ecological preferences, geographic ranges, and taxonomic associations and are most prevalent among Neotropical montane, stream-associated species. The lack of conservation remedies for these poorly understood declines means that hundreds of amphibian species now face extinction.

Scientists first became concerned about widespread amphibian population declines when they met in 1989 at the First World Congress of Herpetology. Historical data indicate that declines began as early as the 1970s in the western United States (1, 2), Puerto Rico (3), and northeastern Australia (4). Subsequent reports revealed the severity of the declines. At one site in Costa Rica, 40% of the amphibian fauna disappeared over a short period in the late 1980s (5). Sudden disappearances of montane species were noted simultaneously in Costa Rica, Ecuador, and Venezuela (5–8). In some regions, many declines took place in seemingly pristine habitats (1–8). These reports were initially received with some skepticism because amphibian populations often fluctuate widely (9), but tests of probabilistic null models showed that the declines were far more widespread and severe than would be expected under normal conditions of demographic variation (5). This finding, in addition to many further reports of declines in the 1990s (8, 10–13), was pivotal in convincing most herpetologists that amphibian declines are nonrandom unidirectional events.

The lack of a comprehensive picture of the extent and severity of amphibian declines prompted us to conduct the IUCN–The World Conservation Union Global Amphibian Assessment (GAA) to gather data on the distribution, abundance, population trends, habitat associations, and threats for all 5743 described species of amphibians (14, 15). From this information, we used the IUCN

Red List Criteria (16) to determine the level of threat to every species. The raw GAA data are publicly available (14). The results demonstrate that amphibians are far more threatened than either birds (17) or mammals (18), with 1856 species (32.5%) being globally threatened [that is, listed in the IUCN Red List Categories (16) of Vulnerable, Endangered, or Critically Endangered], as compared with 12% of birds (1211 species) (17) and 23% of mammals (1130 species) (18). At least 2468 amphibian species (43.2%) are experiencing some form of population decrease, whereas only 28 (0.5%) are increasing and 1552 (27.2%) are stable; 1661 (29.1%) species have an unknown trend.

Many amphibian species are on the brink of extinction, with 427 species (7.4%) listed as Critically Endangered (CR) (the IUCN category of highest threat), as compared with 179 birds (1.8%) (17) and 184 mammals (3.8%) (18). The level of threat to amphibians is undoubtedly underestimated because 1294 species (22.5%) are too poorly known to assess [Data Deficient (DD)], as compared with only 78 birds (0.8%) (17) and 256 mammals (5.3%) (18). A significant proportion of DD amphibians is likely to be globally threatened. Analysis of trends in population and habitat availability indicates

Table 1. Habitat preferences and biogeographic affinities of rapidly declining and enigmatic-decline amphibians in relation to all amphibian species (15). Rapidly declining species are those that now qualify for listing in a IUCN Red List Category of higher threat than they would have had in 1980. Enigmatic-decline species are rapidly declining species that have shown dramatic declines, even where suitable habitat remains, for reasons that are not fully explained.

Habitat preferences	Total number of species (%)	Number of rapidly declining species (%)	Number of enigmatic-decline species (%)
Forest	4699 (81.8)	365 (82.6)	187 (90.3)***↑
Savanna	487 (8.5)	7 (1.6)***↓	0 (0.0)***↓
Shrubland	814 (14.2)	47 (10.6)*↓	14 (6.8)***↓
Grassland	953 (16.6)	81 (18.3)	39 (18.8)
Flowing water	2650 (46.1)	277 (62.7)***↑	164 (79.2)***↑
Marshes/swamps	760 (13.2)	43 (9.7)*↓	14 (6.8)**↓
Still water bodies	2030 (35.3)	107 (24.2)***↓	28 (13.5)***↓
Artificial terrestrial habitats	1304 (22.7)	40 (9.0)***↓	22 (10.6)***↓
Tropical lowland habitats	3392 (59.1)	212 (48.0)**↓	79 (38.2)***↓
Tropical montane habitats	2714 (47.3)	251 (56.8)***↑	155 (74.9)***↑
Biogeographic realms			
Afrotropical	951 (16.6)	28 (6.3)***↓	1 (0.5)***↓
Australasian/Oceanic	561 (9.8)	36 (8.1)	23 (11.1)
Australia and New Zealand	219 (3.8)	32 (7.2)***↑	23 (11.1)***↑
Indomalayan	938 (16.3)	59 (13.3)	1 (0.5)***↓
Nearctic	331 (5.8)	24 (5.4)	9 (4.3)
Neotropical	2,825 (49.2)	279 (63.1)***↑	174 (84.1)***↑
Palaearctic	451 (7.9)	34 (7.7)	2 (1.0)***↓

¹IUCN Species Survival Commission/Conservation International Center for Applied Biodiversity Science Biodiversity Assessment Unit, 1919 M Street N.W., Washington, DC 20036, USA. ²NatureServe, Apartado 75-5655, Monteverde, Puntarenas, Costa Rica. ³Center for Applied Biodiversity Science, Conservation International, 1919 M Street N.W., Suite 600, Washington, DC 20036, USA.

*To whom correspondence should be addressed. E-mail: s.stuart@conservation.org

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (27). ↑Significantly higher than average; ↓significantly lower than average.