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Acknowledgements. This paper is based upon research carried out by the Combined Prehistoric Expedition, which is jointly sponsored by the Southern Methodist University, the Institute of Archaeology and Ethnology, the Polish Academy of Sciences, and the Geological Survey of Egypt. Fieldwork was partly supported by the grants from the US National Science Foundation. We thank the Egyptian Antiquities Organization and A. Radwan for support and assistance.

Correspondence and requests for materials should be addressed to J.M.M. (e-mail: malville@spot.colorado.edu).

Inbreeding and extinction in a butterfly metapopulation

Ilik Saccheri*, Mikko Kuussaari*, Maaria Kankare*, Pia Vikman*, Wilhelm Fortelius† & Ilkka Hanski*

* *Department of Ecology and Systematics, Division of Population Biology, PO Box 17, 00014 University of Helsinki, Finland*
† *Tvärminne Zoological Station, University of Helsinki, 10900 Hanko, Finland*

It has been proposed that inbreeding contributes to the decline and eventual extinction of small and isolated populations[1,2]. There is ample evidence of fitness reduction due to inbreeding (inbreeding depression) in captivity[3–7] and from a few experimental[8,9] and observational field studies[10,11], but no field studies on natural populations have been conducted to test the proposed effect on extinction. It has been argued that in natural populations the impact of inbreeding depression on population survival will be insignificant in comparison to that of demographic and environmental stochasticity[12,13]. We have now studied the effect of inbreeding on local extinction in a large metapopulation[14] of the Glanville fritillary butterfly (*Melitaea cinxia*)[15]. We found that extinction risk increased significantly with decreasing heterozygosity, an indication of inbreeding[6], even after accounting for the effects of the relevant ecological factors. Larval survival, adult longevity and egg-hatching rate were found to be adversely affected by inbreeding and appear to be the fitness components underlying the relationship between inbreeding and extinction. To our knowledge, this is the first demonstration of an effect of inbreeding on the extinction of natural populations. Our results are particularly relevant to the increasing number of species with small local populations due to habitat loss and fragmentation[16].

The Glanville fritillary metapopulation on the Åland islands in southwest Finland is well suited to the study of factors affecting population extinction[15,17,18]. This metapopulation consists of numerous small, more-or-less isolated, local populations breeding on dry meadows with one or both of the larval host plants, *Plantago lanceolata* and *Veronica spicata*. The Glanville fritillary has a yearly life cycle in northern Europe. Adult butterflies mate and females lay eggs in June; caterpillars feed in conspicuous family groups of 50–250 larvae, which facilitates large-scale censusing; caterpillars diapause from August until March, continue feeding in the spring and pupate in May. We have located about 1,600 suitable meadows, ranging from 6 m^2 to 3 ha in size, within an area of 3,500 km^2. Autumnal surveys have revealed that larvae were present in 524, 401, 384 and 320 meadows in late summer of 1993, 1994, 1995 and 1996, respectively. Local populations can be very small, often consisting of just one sib-group of larvae, the offspring of one pair of butterflies. Consequently, population turnover rate is high, with an average of 200 extinctions and 114 colonizations observed per year. The number of local populations has declined during the study period, probably because of a sequence of unfavourable summers.

Populations were characterized between 1993 and 1995 in terms of size (number of larval groups) and isolation (distances to and the sizes of neighbouring populations[19]). Female butterflies were caught in June 1996 from 42 local populations across Åland (Fig. 1), chosen to include relatively large (≥5 larval groups), non-isolated populations (from which 5–10 females were sampled per population), as well as small (<5 larval groups) and isolated populations (from which two females were usually sampled per population).

Individual heterozygosity was determined at seven polymorphic enzyme loci and one polymorphic microsatellite locus (see Methods). The number of heterozygous loci per female was normally distributed, ranging from zero to seven. Heterozygosity differed significantly among the populations ($P = 0.02$). A significant fraction (19%) of variance in heterozygosity among populations was explained by population size in 1993 and by longitude. Heterozygosity was low in populations that had been small in 1993 and in those in eastern Åland. The latter effect apparently reflects large-scale regional changes in abundance in the past[18,20].

Accuracy of heterozygosity as a relative measure of inbreeding is largely dependent on the number and degree of polymorphism of markers used to estimate heterozygosity as well as the magnitude of the differences in inbreeding being measured. The variance in inbreeding among populations is expected to be high in this metapopulation, because there is substantial gene flow in many dense regional networks of local populations[21], but also close inbreeding in many local populations that are extremely small and quite isolated. Thus, differences in average heterozygosity of local populations, even if based on a limited number of polymorphic loci, should reflect real differences in the degree of inbreeding.

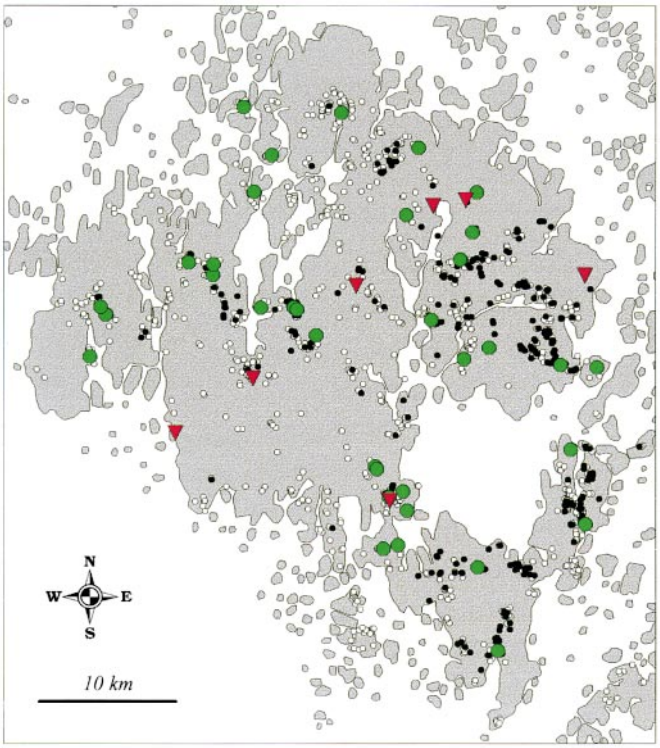

Figure 1 Map of Åland in southwestern Finland showing the locations of the 42 local populations from which adult female butterflies were sampled in summer 1996 (large symbols). All known suitable meadows are shown as small circles, with meadows in which Glanville fritillary larvae were present in autumn 1995 indicated by black circles (and large symbols), and unoccupied meadows by white circles. Of the 42 local populations sampled, the 35 that survived to autumn 1996 (green circles) are distinguished from the seven that went extinct (red triangles).

Seven of the 42 populations went extinct between late summer 1995 and late summer 1996 (Fig. 1). To explain these extinctions, we initially constructed a logistic regression model for the extinction events using population heterozygosity (average number of heterozygous loci per individual) as the explanatory variable (weighted by sample size). The effect of heterozygosity was highly significant ($P > 0.001$). This is unlikely to be the best model for extinction events, however, because many demographic and environmental factors are known to significantly affect extinction risk in the Glanville fritillary[17,18,22]. Furthermore, heterozygosity may be correlated with these other factors.

We therefore used a model previously developed for extinction events in the entire Åland islands between 1993 and 1994 (ref. 17). In this ('global') model, the risk of extinction increased with decreasing population size (log N_{1993}), with decreasing density of butterflies in the neighbourhood of the focal population (N_{neigh}), with decreasing regional trend in butterfly density (N_{trend}), with decreasing habitat patch size (log area), and with the incidence of cattle grazing (grazing)[17]. We now re-estimated the parameters of this model using the observed extinction events between 1995 and 1996, but excluding the 42 populations from which the genetic data had been collected. The remaining material consisted of 336 local populations in 1995, 185 of which went extinct by 1996. In a logistic regression model, log N_{1995}, N_{trend} and log area had a significant effect on the extinction risk. These are also the three variables that had the strongest effect on extinctions between 1993 and 1994 (ref. 17). Using the parameter values obtained for these three variables, we applied the model to the 42 populations with genetic information, also adding population heterozygosity to the model. The observed extinctions were significantly explained by population heterozygosity in this model ('global model' in Table 1; see also Fig. 2).

An important advantage of this analysis is that the effects of the ecological variables were parameterized using an independent data set. A more accurate prediction can be obtained by directly fitting a model to the actual data from the sample of 42 populations. The 42 populations included more large populations than the remaining 336 populations, which is reflected in the much lower incidence of extinctions in the 42 populations (17% versus 55%). Fitting the original model to the 42 populations, N_{trend} and log area had no significant effect, whereas log N_{1995} and N_{neigh} did. As the abundance of nectar-flowers affects immigration and emigration in the Glanville fritillary[23], we included this variable in the model and found that it significantly influenced extinctions in the predicted direction (increased extinction risk with decreasing abundance of flowers). Finally, we added the estimated population heterozygosity to this model and found that it too had a highly significant effect ('sample model' in Table 1), accounting for 26% of the total deviance. In

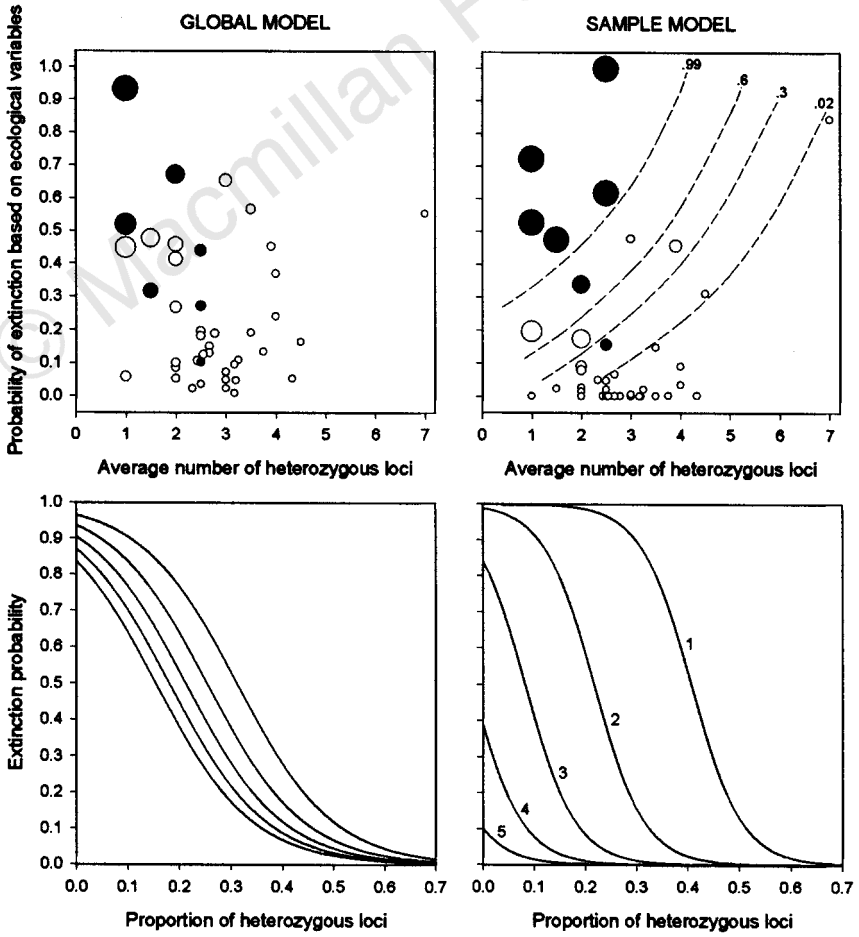

Figure 2 For both global and sample models (Table 1), the upper panels show: (1) the observed average number of heterozygous loci in extinct (black) and surviving (white) populations; (2) the probability of extinction predicted by the models without heterozygosity compared with the observed heterozygosity; (3) the probability of extinction predicted by the full model, including heterozygosity (proportional to circle size). For the sample model, we have drawn appropriate isoclines for the extinction risk predicted by the model, including ecological factors and heterozygosity. These figures illustrate that both the ecological factors and heterozygosity influence the extinction risk (for statistical analysis, see Table 1). Lower panels show the relationship between the risk of local extinction and heterozygosity predicted by the global and sample models (Table 1). Model predictions are shown for local population sizes of 1–5 larval groups, fixed at the lower quartile value of change in regional density (N_{trend}) and the lower quartile value of meadow area in the global model; and fixed at the lower quartile value of regional density (N_{neigh}) and median flower abundance in the sample model.

Table 1 Two logistic regression models for extinction events in the Glanville fritillary between 1995 and 1996

	Global model				Sample model			
Variable	Coefficient (s.e.)	d.f.	Deviance	*P*	Coefficient (s.e.)	d.f.	Deviance	*P*
Constant	5.15 (1.27)				19.89 (9.25)			
Log (N_{1995})	−3.52 (0.61)				−21.90 (10.30)	1	20.02	<0.001
N_{trend}	−3.00 (0.52)							
N_{neigh}					−10.30 (5.62)	1	17.19	<0.001
Log (area)	−0.41 (0.19)							
Flower abundance					−32.30 (17.40)	1	9.68	<0.01
Heterozygosity	−1.33 (0.53)	1	9.61	<0.005	−2.54 (1.12)	1	21.77	<0.001
Residual		40	50.64			37	15.43	
Total		41	60.25			41	84.10	

In the global model the coefficients for variables other than heterozygosity were estimated from the entire metapopulation excluding the 42 populations with genetic data. The global model is the same as previously published[17] for extinctions between 1993 and 1994 but it was re-parameterized for the present material. Variables with a non-significant effect were omitted from the models (the result was essentially the same if all ecological variables used in the previous study[17] were included in the global model). Because the global and sample models contain different explanatory variables, the magnitude of the coefficients for common variables are not directly comparable.

summary, in all three analyses the more heterozygous populations had a lower risk of extinction than the less heterozygous populations.

The relationship between the risk of local extinction and heterozygosity predicted by the global and sample models is shown in Fig. 2. The effect of heterozygosity on extinction risk is most pronounced in small, isolated populations, but even at intermediate levels of isolation (N_{neigh}) extinction risk increases dramatically with decreasing heterozygosity in the smallest populations.

Preliminary findings suggest three major fitness components underlying the observed relationship between heterozygosity and extinction risk in the Glanville fritillary: larval survival, adult longevity and egg hatching. Larval group size shortly after winter diapause was positively associated with maternal heterozygosity (Spearman rank correlation $= 0.32$, $n = 42$, $P < 0.05$), as was larval weight ($P = 0.004$), suggesting that overall larval viability is enhanced in more heterozygous families (details in Methods). Pupal period was negatively correlated with maternal heterozygosity ($P = 0.04$). In natural populations, a longer pupal period is likely to be associated with reduced survival due to parasitism[24].

There was a positive association ($P < 0.05$) between the date females were sampled in the field (from 15 June to 2 July) and their heterozygosity, indicating that females with a shorter life span were more homozygous and inbred. As females are capable of producing a lifetime total of up to seven batches of 50–300 eggs[25], which they are constrained to lay on different days when weather conditions are favourable, a small reduction in life expectancy due to inbreeding may have large effects on reproductive output and therefore on population dynamics.

Laboratory studies of the Glanville fritillary from Åland have shown that one generation of brother–sister mating reduces average egg-hatching rate by 24–46% (W.F. *et al.*, unpublished results; see Methods), indicating that the metapopulation has remained sensitive to inbreeding in spite of frequent episodes of local inbreeding in the often very small populations, where sib-mating must occur frequently. In the 42 populations sampled, variance in average egg-hatching rate (determined for one to three egg clutches per female) was significantly larger among populations with low heterozygosity ($\leq$ two heterozygous loci per individual) than in populations with high heterozygosity ($\geq$ three heterozygous loci) (Bartlett's test of equal variances, $P = 0.007$). This was essentially due to a low egg-hatching rate in several populations with low heterozygosity and is an expected outcome of both inbreeding and the interaction of inbreeding and selection[6,7].

Detection of the effect of inbreeding on extinction risk was possible in this study by virtue of numerous extinction-prone local populations varying in their degree of inbreeding. Our results suggest that the Glanville fritillary metapopulation maintains a high genetic load, making it susceptible to inbreeding depression. Selection against deleterious recessives exposed by localized inbreeding may be relatively inefficient owing to drift within[26,27] and gene flow among neighbouring small local populations that carry different deleterious alleles. This little-studied aspect of metapopulation biology may have far-reaching consequences for the expected persistence times of fragmented populations. The general message from this study is that, although demographic and environmental factors are likely to be the primary determinants of extinction risk, the contribution of inbreeding should not be underestimated, especially in species with a highly fragmented population structure. □

Methods

Measurement of heterozygosity. Cellulose acetate electrophoresis was used to assay polymorphism in the following enzymes: *Ak*, *Got-1*, *Idh-1*, *Pep A*, *Pep D*, *Pgi* and *Pgm*. The microsatellite locus used in this study (CINX22) was cloned from a partial genomic DNA library of *M. cinxia*. The number of alleles and observed heterozygosity for each locus were: 3, 0.29 (*Ak*); 2, 0.18 (*Got-1*); 3, 0.39 (*Idh*-1); 9, 0.57 (*Pep A*); 3, 0.24 (*Pep D*); 7, 0.57 (*Gpi*); 2, 0.3 (*Pgm*); 5, 0.57 (CINX22).

Larval survival and growth. One larval group from each wild-caught female was returned to the meadow of origin to minimize the impact of sampling on population dynamics and to monitor larval survival. A second larval group from most females was placed on a large previously unoccupied meadow on an isolated island to study larval development under common environmental conditions. Larval group size was determined in April 1997, shortly after the caterpillars had come out of winter diapause. The significant correlation between larval group size and heterozygosity refers to 103 groups from 42 populations returned to the meadow from which the mother was caught (correlation was calculated for population averages). Larval group size could not be reliably estimated for groups placed in the common meadow owing to starvation and dispersal in many groups caused by local food shortage. Larval groups that had not been badly affected (> ten larvae survived) in the common meadow were collected and weighed. Linear regression showed a significant positive relationship between the average weight of offspring and maternal heterozygosity: $F_{1,22} = 10.46$, $P = 0.004$, $R^2 = 0.30$. The same larvae were reared to adulthood, providing data on larval growth rate, pupal weight and pupal period (S. Haikola, unpublished results).

Female heterozygosity and sampling date. In the generalized linear model of heterozygosity on sampling date, differences in sampling date among populations were accounted for by fitting population means before adding heterozygosity to the model.

Egg-hatching rate. Egg batches collected from individual females were counted from an enlarged photocopy after they had been spread out on a Petri dish. Following an incubation period of 14–21 days at 22–26 °C and 70–90% relative humidity, a second photocopy was taken from which larvae could be counted. The estimates of the effects of inbreeding on egg-hatching rate reported here come from two laboratory experiments conducted at different times using independent samples of butterflies (W.F. *et al.*, unpublished results). In the first experiment (spring 1997), the average egg-hatching rate in six brother–sister matings, originating from five unrelated families from four distant local populations, was 37%, compared to 69% in 12 replicated pairwise interpopulation crosses. The butterflies used in the second experiment (summer 1997) were the F_2 generation from 13 wild families from three distant populations, which were in some cases the product of brother–sister

matings. Average egg-hatching rate in 64 full-sib matings representing all founder families was 60%, compared to 79% in 48 non-sib crosses within and between populations.

Received 22 September 1997; accepted 4 February 1998.

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Acknowledgements. We thank K. Higgins, L. Keller, R. Lande, M. Lynch, A. Moilanen, R. Nichols, S. van Nouhuys, M. Singer and C. Thomas for their helpful comments on the manuscript.

Correspondence and requests for materials should be addressed to I.S. (e-mail: ilik.saccheri@helsinki.fi).

Cerebellar complex spikes encode both destinations and errors in arm movements

Shigeru Kitazawa*†, Tatsuya Kimura* & Ping-Bo Yin§

** Information Science Division, Electrotechnical Laboratory,*
† PRESTO and § CREST, Japan Science and Technology Corporation, 1-1-4 Umezono, Tsukuba 305-8568, Japan

Purkinje cells of the cerebellum discharge complex spikes, named after the complexity of their waveforms[1], with a frequency of ~1 Hz during arm movements[1–13]. Despite the low frequency of firing, complex spikes have been proposed to contribute to the initiation of arm movements[2,7–10] or to the gradual improvement of motor skills[2,4–6,14–16]. Here we recorded the activity of Purkinje cells from the hemisphere of cerebellar lobules IV–VI while trained monkeys made short-lasting reaching movements (of ~200 milliseconds in duration) to touch a visual target that appeared at a random location on a tangent screen. We examined the relationship between complex-spike discharges and the absolute touch position, and between complex-spike discharges and relative errors in touching the screen. We used information theory to show that the complex spikes occurring at the beginning of the reach movement encode the absolute destination of the reach, and the complex spikes occurring at the end of the short-lasting movements encode the relative errors. Thus, complex spikes convey multiple types of information, consistent with the idea that they contribute both to the generation of movements and to the gradual, long-term improvement of these movements.

Two macaque monkeys were trained to make rapid reaching movements toward a visual target that appeared on a tangent screen, located 200 mm from the eyes, from a button positioned 200 mm below the eyes in the mid-sagittal plane. A trial began when the monkey pressed the button (Start, Fig. 1); a target then appeared (Target, Fig. 1) at a random place in a square target zone (50×50 mm, or 80×80 mm) on the screen. The monkey had to release the button (Release, Fig. 1) within 240 ms of the appearance of the target and touch the screen within 300 ms of releasing the button (Touch, Fig. 1). The monkey's view of its hand and the target was blocked at the release of the button by liquid-crystal shutters in front of the eyes (Release, Fig. 1). The shutters opened again when the screen was touched (Open, Fig. 1), allowing the monkey to see the target and the final position of its hand for 300 ms. The monkey had to hold the final position of its hand for 900 ms until given a reward (Reward, Fig. 1); the size of the reward was in inverse proportion to the magnitude of the error to encourage accurate reaching.

Figure 2 shows a raster plot (Fig. 2a) and the average discharge frequencies (Fig. 2b) of a Purkinje cell from lobule V. Stable simple spikes and complex spikes were recorded from this Purkinje cell during 1,381 trials. The average discharge frequency of simple spikes, (Fig. 2b) decreased to 40 Hz during the movement, and then sharply increased to 170 Hz at the end of the movement (Touch, time zero). In contrast, complex spikes generally occurred only once during each trial (black dots, Fig. 2a) and the average discharge frequency was less sharply altered during the movement (Fig. 2b). To test whether the sporadic complex-spike discharges of this Purkinje cell ever encode absolute destination of the reaching movement or relative errors, we set three time windows (shown with crossed, diagonal or horizontal lines in Fig. 2b).

During the first time window in the first half of the movement (crosshatched area in Fig. 2b), complex-spike discharges occurred in 133 of the 1,381 trials. Figure 3a shows the absolute position of

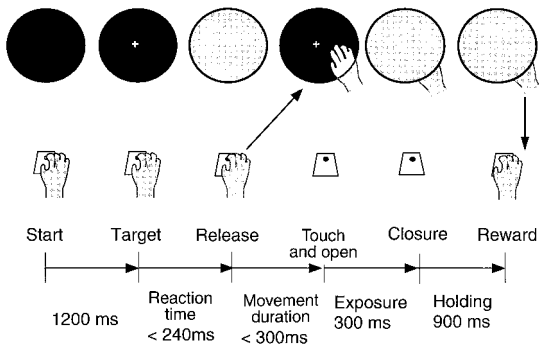

Figure 1 The reaching task. The sequence of events for each trial is shown from left to right. A trial begins when a monkey touches a button. A target beam then appears on the screen. The monkey then releases the button, and touches the target on the screen. At this point, liquid-crystal shutters that were obscuring the monkey's view of its hand are opened for 300 ms, then closed. The monkey holds the position of its hand on the target for 900 ms, and then receives a reward.

Beyond the semantics raised by these hotly debated proposals lurks the problem of how to compare fitness differences across levels of selection, a problem that is unavoidable if one wants to understand the organization of a genome able to modify its own mutation rate (B. Godelle, Univ. Paris XI)[7] or the transition from unicellular to multicellular eukaryotes — a transition that must cause smouldering conflicts within the developmental system of the emerging entity (R. Michod, Univ. Arizona).

A careful analysis of symbiosis and mutualism stresses that analysis at different levels — physiological, ecological and evolutionary — may lead to different conclusions (U. Dieckmann, Intl Inst. Appl. Syst. Anal.; R. Law, Univ. York). Even in the face of persistent physiological exploitation of one partner by the other, evolution can select for stable symbiotic structures: such adaptations lead to a kind of dependence that is more like addiction than mutual benefit.

This may shed light upon the origin of mitochondria in eukaryotic cells. Suggestions favouring a very early acquisition of mitochondria suffer from two unsolved problems: the method of acquisition (no sensible alternative to phagocytosis has ever been suggested), and the initial advantage of such an association. Perhaps proto-mitochondria were once parasitic, and only later evolved into ATP-generating slaves. As one of us (E.S.) pointed out, isogametic sex may have been crucial, as it allows the spread of moderately harmful intracellular symbionts.

Scenarios of this type vastly expand the range of conditions under which separate lineages can be expected to merge into symbiotic units. This leads one to hope, on yet another level, that mathematicians and biologists will find their emerging association of mutual benefit. They may eventually become addicted to each other's company. □

Karl Sigmund is at the Institute for Mathematics, University of Vienna, Strudlhofgasse 4, A-1090 Vienna, Austria, and the International Institute for Applied Systems Analysis, A-2361 Laxenburg, Austria.
e-mail: ksigmund@esi.ac.at
Eörs Szathmáry is in the Department of Plant Taxonomy and Ecology, Eötvos University, Budapest, and the Collegium Budapest, Szentharomsag u. 2., H-1014 Budapest, Hungary.
e-mail: szathmary@colbud.hu

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Conservation biology

Inbreeding leads to extinction

Richard Frankham and Katherine Ralls

Do genetic problems contribute to the endangerment and extinction of wild populations? Conservation biologists initially thought[1] that they would — and seriously so. But it is extremely difficult to demonstrate that inbreeding contributes to the extinction of wild populations. On page 491 of this issue, however, Saccheri and colleagues[2] provide the first direct evidence that it does, with their elegant work on a wild butterfly metapopulation in Finland.

Theoretical work in the 1980s indicated that small populations in the wild suffer from increased extinction because of an unavoidable increase in matings between close relatives. Inbreeding reduces reproductive success in populations of naturally outbreeding species, both in captivity[3,4] and in the wild[4], and it also increases extinction rates in laboratory populations of fruitflies and mice[5]. However, in an influential paper[6], Lande argued that random demographic and environmental events will drive small wild populations to extinction before genetic factors come into play. Environmental events, ranging from annual variation in climatic variables (such as rainfall) to catastrophes (such as disease epidemics), do increase the probability of extinction. Furthermore, inbreeding typically interacts with demography by reducing fecundity, juvenile survival and lifespan. Because there is no direct evidence that inbreeding contributes to extinction of wild populations, some researchers have continued to question the relevance of genetic factors[7,8].

The Glanville fritillary butterfly (*Melitaea cinxia*; Fig. 1) studied by Saccheri *et al.*[2] has a predictable yearly life cycle. Adults emerge, mate, and lay eggs in June. Caterpillars feed in conspicuous family groups of 50 to 250 individuals, then diapause (suspend development) from August until March of the following year, and resume feeding and pupate in May. The butterfly metapopulation consists of numerous small populations that breed in about 1,600 suitable dry meadows of different size and varying distance from one another. Some populations are very small, often consisting of the offspring of a single pair of butterflies. Consequently, populations in individual meadows often disappear, but many meadows are eventually recolonized, with an average of 200 extinctions and 114 colonizations per year.

Because small population size results in both inbreeding and loss of genetic variation, the degree of genetic variation in a population serves as a measure of the extent to which it is inbred. Saccheri *et al.* determined the genotypes of female butterflies from 42 populations at eight variable genetic loci (polymorphic loci). They sampled relatively large, non-isolated populations, as well as smaller, relatively isolated populations. The authors found that populations with less genetic variation were more likely to become extinct. Furthermore, multiple logistic regression showed that genetic diversity predicted extinction risk after accounting for all known demographic, ecological and environmental causes of extinction in this well-studied butterfly metapopulation. Inbreeding reduced the egg hatching rate and larval survival, lengthened the dura-

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Figure 1 **Doomed liaison — a mating pair of Glanville fritillary butterflies (*Melitaea cinxia*). From their studies of a metapopulation of this species, Saccheri *et al.*[2] found that inbreeding contributes to the extinction of wild populations.**

tion of the pupal period (so that inbred pupae were more likely to be parasitized), and shortened female lifespan (so that inbred females tended to lay fewer eggs). Overall, inbreeding explained 26% of the variation in extinction rate among the butterfly populations.

Several indirect lines of evidence imply that these results from the Glanville fritillary butterfly can be extended to other species. First, theoretical studies have shown that genetic factors probably contribute to extinctions, even when demographic and environmental fluctuations and catastrophes are operating[9]. Second, genetics may be a factor that makes island populations prone to extinction. Although humans (and the animals that they have introduced) have decimated many island populations, these island populations have lower genetic diversity than mainland populations[10]. Moreover, many are inbred to levels where captive populations show an increased risk of extinction from inbreeding[11]. Third, ratios of effective-to-census population sizes seem to be much lower than suspected[12], so genetic concerns become more important in larger populations than previously believed. Fourth, endangered species tend to have lower genetic diversity than non-endangered species[4] — this would not be expected if ecological factors drove populations to extinction before genetic factors became important. Finally, the extinction rate of a wild plant was higher in experimental populations with low versus higher genetic variation when both were planted in the field[13].

It is hard to escape the conclusion that genetic factors are involved in the extinction of wild populations. Consequently, genetic factors must be considered when assessing endangerment and devising recovery plans for threatened species. □

Richard Frankham is in the School of Biological Sciences, Macquarie University, New South Wales 2109, Australia.
e-mail: rfrankha@rna.bio.mq.edu.au
Katherine Ralls is in the Department of Zoological Research, National Zoological Park, Smithsonian Institution, Washington DC 20008, USA.
e-mail: rallsk@thegrid.net

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Apoptosis

Phagocytic docking without shocking

John Savill

Apoptosis, a programmed form of cell death central to health and disease, is both topical and tidy. Doomed cells are swiftly identified and engulfed by phagocytes. But what are the mechanisms concerned? Answers on two fronts come in the papers on pages 501 and 505 of this issue by Wu and Horvitz[1] and Devitt *et al.*[2].

In normal tissues apoptosis was overlooked for many years because it is inconspicuous — intact dying cells are recognized, ingested and degraded beyond histological recognition by scavenger cells[3]. These can be neighbours acting as 'semi-professional' phagocytes or voracious experts of the macrophage line. Phagocyte recognition of 'apoptotic self' is also essential in protecting tissues from inflammatory injury due to leakage of noxious contents from dying cells[4]. During apoptosis these are safely packaged into membrane-bound bodies tagged with 'eat me' signals such as exposed phosphatidylserine[5]. However, there has been remarkably little study of how apoptotic cells reach their unmarked graves within phagocytes. Hence the value of the new work, on the nematode worm, *Caenorhabditis elegans*[1], and human cells[2], respectively.

Caenorhabditis elegans must be a strong candidate for the 'most valued player' award in apoptosis research. Mutations affecting developmental cell deaths in this worm identify *ced* (cell death abnormal) genes which are homologous to, and sometimes interchangeable with, key elements in human apoptosis[6]. At least six genes regulate the semi-professional engulfment of cell corpses in *C. elegans*, but *ced-2*, *ced-5* and *ced-10* mutants also exhibit a fascinating and specific defect in migration of the distal tip cells of the gonad.

Wu and Horvitz[1] have now cloned and sequenced *ced-5*. They have discovered that this gene encodes a protein similar to DOCK180, a human cytoplasmic molecule which bears an SH3-domain 'passport' that allows it to interact with signalling pathways[7]. Interestingly, farnesylated DOCK180 can drive cell spreading, implying that it is involved in the regulation of cell movement by tyrosine kinases. Furthermore, the authors[1] found that DOCK180 rescued distal-tip-cell migration in a *ced-5* mutant. It was perhaps too much to expect concomitant rescue of the engulfment defect; nevertheless, this was achieved by regulated expression of wild-type CED-5 protein, apparently through its effects on engulfing cells. Emboldened by the sequence similarities between CED-5, DOCK180 and the *Drosophila* protein Myoblast City, which is implicated in myoblast fusion[8], Wu and Horvitz make the provocative suggestion that CED-5 is involved in the cytoskeletal reorganization required for an engulfing cell to extend its surface around a dying cell during phagocytosis.

Which phagocyte transmembrane molecules might trigger CED-5-mediated 'interment' of an apoptotic cell? The complete sequences of the five other *ced* genes involved in engulfment have yet to be published, but candidate molecules have been provided by a cell biological rather than a genetic approach. The first clues came from a simple *in vitro* model[9], in which monosaccharides inhibited macrophage binding of apoptotic thymocytes, implicating phagocyte lectins such as the asialoglycoprotein receptor in recognition of sugar changes on apoptotic cells.

By backing hunches, other cell biologists used a similar inhibitor-based approach *in vitro* to identify further suspects — class A scavenger receptors[10], a distinct scavenger receptor now identified as macrosialin[11], and ABC1, a mammalian ABC transporter[12], which may represent a homologue of the *C. elegans* engulfment gene *ced-7*. Two multi-functional adhesion molecules that bind bridging thrombospondin were also implicated in phagocyte recognition of apoptotic cells in culture. These were the $\alpha_v\beta_3$ vitronectin receptor integrin[13] and the class B scavenger receptor CD36 (ref. 14), the index member of a family of phagocytic molecules that includes human CLA-1 and *Drosophila* Croquemort. Because $\alpha_v\beta_3$ and CD36 can signal via tyrosine kinases, these receptors could interact with CED-5-like molecules and the phagocyte cytoskeleton in promoting engulfment. Figure 1 shows a line-up of the molecules thought to be involved.

Could there be 'tethering' receptors which, by virtue of their high mobility within the phagocyte plasma membrane, capture apoptotic cells and shuttle them to the phagocytic machinery? One painstaking and systematic approach to this question involved screening a huge number of previously generated monoclonal antibodies in an assay of apoptotic lymphocyte tethering to macrophages. Most notably, the unclassified monoclonal antibody 61D3 blocked tethering and phagocytosis[15].

In the second paper in this issue, Devitt *et al.*[2] now report expression cloning of the