

F-3.1.3 Studies on survival and reproduction of animals in relation to genetic variability

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Abstract Genetic stochasticity is one of many potential causes of extinction in small populations. Objects of this research are to know how genetic variability changes according to the reduction of population size or habitat destruction, and how the decrease in genetic variability influences the reproductive output of animals. In the process of population decrease, populations may suffer a significant genetic deterioration, namely a decrease in genetic variability, which may lead to a corresponding decrease in fitness (survival and reproduction). As Fitness can often be measured as fluctuating asymmetry (FA), we analysed FA in relation to various fitness-related characters, such as survival, growth rate, territoriality and/or reproductive success in several animals. We found negative correlation between mating success and FA in insects including dragonflies, butterflies and midges. FA measurements on several passerine birds revealed that population density and the distribution ranges were negatively correlated with population mean of FA.

Key Words small population, genetic variability, fitness, fluctuating asymmetry

1. Introduction

Genetic diversity can be viewed as occurring at four levels of organisation: among species, among populations, within populations, and within individuals. Usually we can easily distinguish genetic differences from one species to another even without any knowledge about their genes. Genetic diversity among the populations of a single species is common but is usually not revealed in conspicuous characters, unless they are taken from geographically distant localities. Within populations of most species, all individuals look very similar, but each individual is genetically different, more or less, from each other. Genetic diversity exists even within a single individual when there are two or more alleles occupying the same locus on a chromosome. From the view point of wildlife conservation, we should focus on the level of genetic diversity within populations.

Genetic diversity can be measured based on the distribution of different alleles among individuals and can be expressed as polymorphism (based on the proportion of genes which have more than one common allele) or heterozygosity (based on the proportion of genes for which the average individual is heterozygous). Why is it important to maintain genetic diversity within populations? In other words, why should different versions of the same gene be well distributed in a population; why should heterozygotes be favoured rather than homozygotes? There are two basic answers to this question in relation to population viability. (1) Populations with greater genetic diversity are more likely to evolve in response to a changing environment than those with less diversity. (2) Populations that lack genetic diversity may also suffer low fitness (low fertility, high mortality, etc.) even in environments that are not changing. The first

answer is comparatively simple and well documented in a number of animals and plants, however, the mechanism of the second phenomenon is still open to several explanations (expression of recessive deleterious alleles, over dominance, etc.).

2. Processes that diminish genetic diversity

Genetic diversity can be diminished by three phenomena associated with small populations. First, when a population is reduced to a small size, some genetic variance and rare genes are likely to be lost. Second, among small populations, especially those that remain small for many generations, random genetic drift changes the frequency of alleles, often reducing genetic diversity or even genes to be fixed for a single allele. Third, inbreeding between closely related individuals can diminish genetic diversity.

To investigate the difference in genetic diversity among populations with different degrees of inbreeding and the effects of small population size, we used Random Amplified Polymorphic DNA (RAPD) analysis conducted on Medaka-fish (*Oryzias latipes*). Using purified DNA, random fragments are amplified using the polymerase chain reaction (PCR). Following electrophoresis, nucleic acids are stained and diagnostic bands are determined.

Medaka-fish was found almost everywhere in rice fields throughout Japan and was a popular fish. However, it is becoming rarer and rarer probably due to pesticide usage and recent alteration of water supply systems in rice fields. On the other hand, this fish species has long been used in the study of genetics. Consequently, many pure genetic lines of medaka-fish have been kept in various institutes or universities in Japan. In addition, medaka-fish have been popular as pets: we can easily buy this fish from a pet shop. They are not genetically homogeneous, but must be less variable than wild fish because they have been bred, no doubt, from a few individuals. As a result, we could easily obtain populations with three different degrees of small population effects for our genetic analysis. As we expected, electrophoresis revealed that the wild population of medaka-fish showed high levels of genetic variation, while highly inbred population (HO5) showed little genetic variation. Another population obtained from a pet shop showed intermediate levels.

3. Fluctuating asymmetry as measure of fitness

Fluctuating asymmetry (FA) is minor non-directional deviations from bilateral symmetry in morphological characters. FA is widely used as a measure of developmental stability and usually increases with increasing environmental or genetic stress. Deviations from symmetry often correlate with fitness differences. Departures from symmetry are most commonly described by frequency distributions of right - left (R - L). Comparison of the degree of FA for medaka-fish among three populations (wild, intermediate and highly inbred HO5 populations) revealed that wild population showed smallest degree of FA and HO5 showed highest FA (Fig. 1). The character measured here is pectoral-fin length. These results suggest that genetic diversity within populations stabilises development by some unknown mechanism buffering against environmental noise, and possibly increases the viability of populations.

The study of FA is a potentially valuable and inexpensive tool for detecting stress in natural and captive populations, although it will not be useful for all species or in all situations. Further studies should be made to determine which species and which characters may be useful indicators of stresses associated with population decline.

4. Fluctuating assymetry variation within populations

FA varies not only among populations but also among individuals within a population. In the medaka-fish, we found a negative correlation between individual growth rate and FA in pectoral fin length within a cohort population. That is, larger individuals tended to show smaller FA

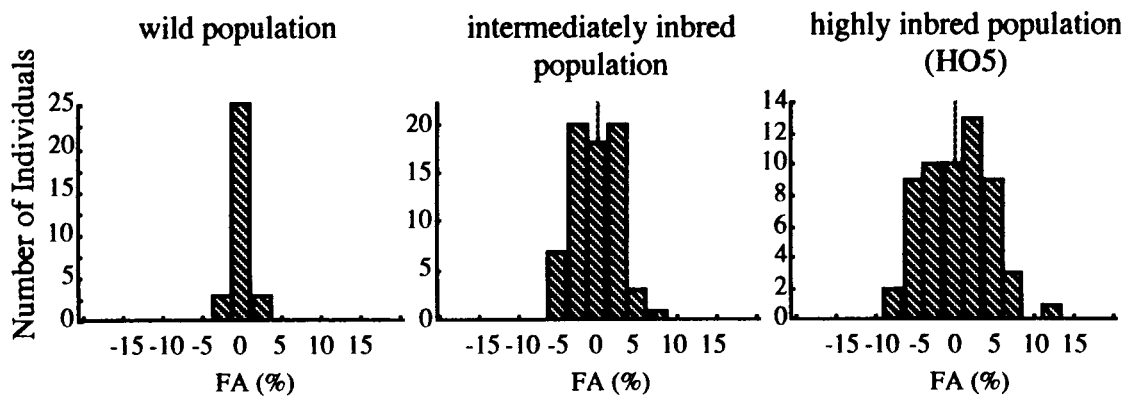


Fig. 1. Distribution of fluctuating asymmetry (FA) values for three populations of medaka-fish (*Oryzias latipes*). Character measured was pectoral-fin length. As the average size was different between populations, FA was shown as a proportion of the average of right and left pectoral-fin lengths.

than smaller ones. This phenomena may be explained in two ways. First, there exist a variation in individual quality which is genetically governed, and was shown both in FA and growth rate. Second, environmental stresses, such as intraspecific competition or shortage of food and space during growth periods, disturbed the normal growth of pectoral fins. To know which is important the genetic stress or the environmental one, we conducted a laboratory experiment to detect the density effects on pectoral fin FA. As a result, we could not detect any density effects on FA, though the density had a clear negative effects on body size.

5. Fluctuating asymmetry as a measure of reproductive success

How the degree of FA and other morphological characters correlate with mating success of males was studied on a Papilionid butterfly (*Luehdorfia japonica*). When a male was captured in the field, we could estimate how many times he had copulated before the capture by examining the amount of hair left on his abdomen. Length of 13 wing veins were measured to

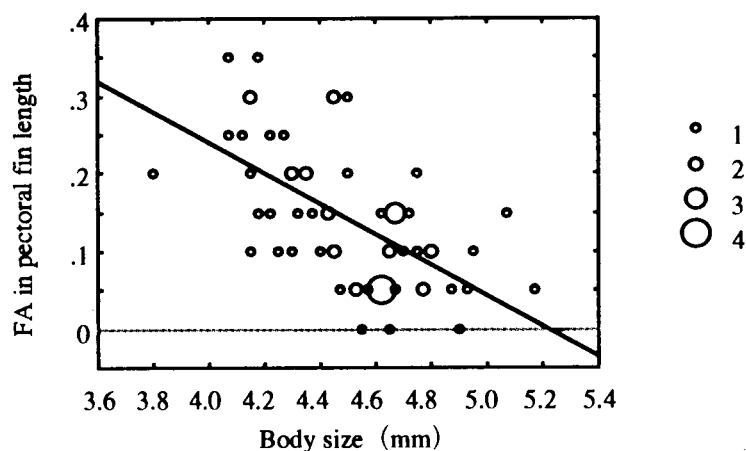


Fig. 2. Fluctuating asymmetry in relation to the body size (measured as right and left average of pectoral fin length) of medaka-fish (*Oryzias latipes*) within a cohort population.

estimate mean FA as a measure of quality of males. Negative correlation was found between the mating frequency and FA, and between age and FA, however, size or wing darkness was not correlated with mating frequency. It was suggested that males with smaller FA tend to live longer and have higher rate of obtaining females than others.

6. Fluctuating asymmetry in Sylvid and Emberizid birds

We analyzed the fluctuating asymmetries of wing and tarsus length in some Sylvid and Emberizid species. Of those species, the Japanese Marsh Warbler *Megarulus pryeri* is categorized in vulnerable species and the Japanese Reed Bunting *Emberiza yessoensis* is categorized into rare species in Japanese Red data list. Other control species are not categorized into any threatened status. The study was conducted at Outsukue-jima islet at Hakata Bay and in the marsh area along Lake Kasumigaura and the Tone River. Mist-netting was carried out for a few consecutive day by means of 10-20 mist-nets. Birds captured were measured tarsus length and wing length of both bilateral side twice by means of digital calipers as accurate as 0.01mm, and all data were automatically stored into data loggers. The absolute indices, $|R-L|$ and $|R-L|/\text{mean}(R+L)/2$, are adopted to indicate the degree of fluctuating asymmetry.

As the level of fluctuating asymmetry was only 2 % of characteristic value, repeatability was calculated from twice measurement to remove measurement error. Wing length and tarsus length were highly repeatable and measurement error is less than 10%. The degree of fluctuating asymmetries in wing length were almost identical across the species, and it did not exceed 2 % of characteristic value. However, that in tarsus length were more variable, and it exceed more than 2% of it in four species and sometimes reached as high as 5% for the great reed warbler *Acrocephalus arundinaceus*. The degree of fluctuating asymmetry of juveniles was larger than adults in the most species, and the proportion of extreme asymmetrical individuals was reducing with increasing age. The degree of fluctuating asymmetry between two characteristic were not correlated each other except for Styan's grasshopper warbler *Locustella pleskei*. It might suffer from strong genetic stress because of small population size

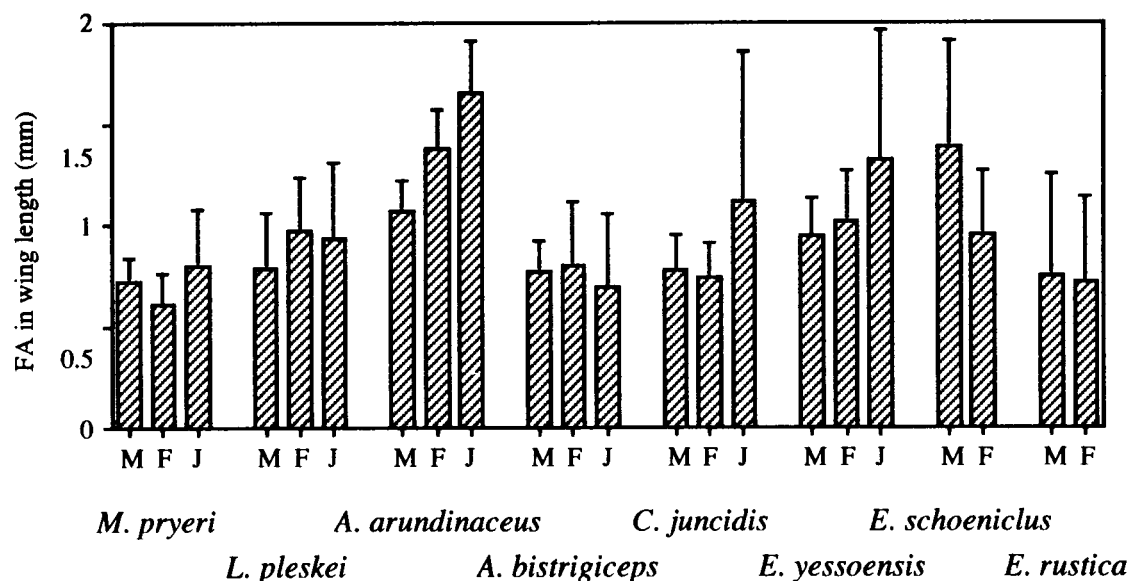


Fig. 3. Average fluctuating asymmetry in wing length for 5 Sylvid and 3 Emberizid bird species. M, F and J indicate males, females and juveniles, respectively.

$N_e=60$. Any consistent tendencies between the degree of fluctuating asymmetry and population parameters could not be found because of the lack of enough information about demographic parameters. Marginal populations were more asymmetrical than central populations in Japanese reed bunting and Japanese marsh warbler. The degree of fluctuating asymmetry was highly variable among reed beds in great reed warblers. Those inhabiting small and isolated reed beds were more asymmetrical because such habitat might give more environmental stress on birds. Fluctuating asymmetry may be effective to evaluate the level of both environmental and genetic stresses.

7. Population structure and genetic variability

Effective population size was estimated by using individual-based models. The simulation results showed that the inbreeding effective population size estimated by the rate of decrease of heterozygosity during the first 15 generations corresponds to the neighbourhood size calculated by the standard deviation of the dispersal distance. However, the inbreeding effective population size estimated by heterozygosity using the first 500 generations is larger than the neighbourhood size calculated by the dispersal distance and increases with the area of simulation with the same densities. The effects of habitat shape on effective population size were also examined. The heterozygosity greatly changed when the probability of occurrence of subpopulations in a lattice environment was 0.5 - 0.6. These individual-based simulations can be easily modified by changing dispersal behaviour and reproductive patterns of the artificial individuals to correspond to values representing the real world. The effect of dispersal behaviour on the effective population size can be assessed by using the individual-based simulations. Such simulations can be useful for both evolutionary biology and conservation biology.