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Inclusion of a genetics component in the simulation model for Kirtland's warblers: recommendations to the Recovery Team

A report for the Kirtland's Warbler Recovery Team

by

Jonathan Bart, Cindy Bronson
Ohio Cooperative Fish and Wildlife Research Unit
Ohio State University
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Summary

The Ohio Cooperative Research Unit is preparing a comprehensive population simulation program for Kirtland's warblers. This report discusses whether the model should include a genetic component. We conclude that the only major genetics issue likely to be of importance to the Kirtland's Warbler Recovery Team is inbreeding depression. Empirical evidence from nesting and banding studies and from the population's recent rapid increase indicate that inbreeding depression is probably not a problem at present. Theoretical and field studies from other species suggest that inbreeding depression could become a problem in the future if "effective population size" for this species is below 500. Determining whether effective population size is less than 500 thus seems worthwhile and should be possible using results from recent field studies on productivity, survival rates, and dispersal behavior. If effective population size turns out to be less than 500 then the Recovery Team might

wish to consider undertaking additional work to estimate the potential future decline in population viability due to inbreeding problems and how it would be affected by different recovery goals. At present, however, we recommend that the simulation model not attempt to make such predictions and that it include a genetics component only for the purpose of helping to estimate effective population size. We provide a brief appendix describing field and laboratory methods for estimating genetic variability in case the Recovery Team is interested in such a study at some future time.

Introduction

The Kirtland's Warbler Recovery Team has asked the Ohio Cooperative Research Unit to prepare a comprehensive computer simulation model that can be used to help develop and evaluate the warbler recovery program. The model will simulate the Kirtland's warbler's population dynamics and will project population size and distribution through periods of several decades under different management scenarios. It will be a "spatially explicit, individually-based model" meaning that it will include a detailed description of the spatial distribution of habitat and how this distribution changes through time, and it will simulate the birth, behavior, and movements of individual birds within the population. These models have proven to be helpful in both theoretical and management investigations and are now being studied by numerous investigators throughout the world. The field is sometimes referred to as "artificial life" because the lives of individual organisms are simulated within the computer.

When the Ohio Cooperative Research Unit proposed developing a Kirtland's warbler model, it expressed some uncertainty over whether genetic factors should be included in the model. Giving each warbler a set of "genes" at the beginning of the simulation, and then keeping track of them during the analysis, is quite easy and probably worthwhile. Deciding whether to make the vital rates (births, deaths, movements) a function of genetic composition, however, is more difficult. Conservation biologists seem to agree that habitat loss and demographic factors generally pose larger risks to threatened and endangered species than genetic problems (e.g., Lande 1988). Furthermore, we were not sure how much basis exists for linking vital rates to genetic factors. On the other hand, we hesitated to exclude genetic factors as a determinant of viability without a thorough review of current literature on the subject. We therefore proposed the preparation of a report analyzing this issue and making a recommendation as to whether the vital rates should be linked to genetic factors. This is that report. We review the management decisions for which the model is being prepared, the possible concerns about genetic issues, and the evidence from theoretical and field studies bearing on these issues that helps indicate whether inclusion of genetic factors in the model might be useful. We assume that each bird will be given one or more genes in the model, because this is easy to do and will permit us to describe the genetic composition of the simulated population and how it changes through time and space.

Management issues which the model may help resolve

Simulation models should be thoroughly evaluated before they are used to make or defend management decisions (e.g., Orlob, 1975, Caswell 1976, Laymon and Barrett 1986). Furthermore, such evaluations must be carried out for each specific prediction because the reliability of different predictions, including ones which initially appear rather similar, may be quite different (Overton 1977). Describing the model predictions of primary interest is thus important as a first step in model building. Some of the management issues which we believe the Team hopes to study using the simulation model are:

1. What level should population size be maintained at (a larger population will be more expensive to maintain than a smaller one)?

2. How should Kirtland's warbler habitat be distributed

within the species' current range?

3. Should a second breeding population be established in the Upper Peninsula or elsewhere?

The genetic issues which might warrant consideration in studying the management questions above include the following:

1. Is the warbler population at risk, or likely to be at risk, from inbreeding?

2. Should the Recovery Team be concerned about preserving existing local adaptations?

3. Should opportunities for genetic divergence be considered in developing a management strategy?

The first topic above, inbreeding, is discussed in some detail in the sections below. The second and third issues amount to asking whether genetically distinct sub-populations either do exist now or might come into existence in the future. If they exist now, and should be preserved, then this goal might have some implications for distribution of breeding habitat in the existing population. Preliminary evidence on dispersal distances (Kepler pers. comm.), however, suggests that the current population is probably well-mixed genetically. Furthermore, the current breeding population occurs in such a small and uniform area that significant local differentiation, at a genetic level, seems unlikely. Both of these issues warrant additional attention (and will be given more attention in the course of our work), but we feel that this genetic issue does not warrant further attention at this time by the Team.

The third issue above, whether genetic differences might come into existence in the future, would probably only be of any relevance if a second breeding population were established. Even if one is established, however, the time frame for any significant local adaptation to develop is probably measured in hundreds or thousands of years. Furthermore, such differentiation might not develop due to genetic exchange with the existing population. Finally, we see no clear way to decide whether such development would be beneficial or detrimental to the overall viability of Kirtland's warblers. For all of these reasons, we feel that the issue of genetic divergence should not be considered further by the Team.

Inbreeding depression

Most populations have a few alleles (genes) which are recessive (i.e., produce effects on the organism only when both chromosomes have the same allele) and have deleterious or lethal effects in homozygous individuals. The average human, for example, probably has three to five lethal recessive genes (Morton et al. 1956). The frequency of each deleterious recessive is low, so few homozygous offspring are produced if mating is random. When close relatives breed, however, both parents may carry the same deleterious allele as a result of common descent (the alleles found in both parents are replicates of one from a common ancestor). Offspring produced by the breeding of close relatives thus have a much greater chance of being homozygous for one or more deleterious alleles and suffering their deleterious effects (Hartl and Clark 1989). Increased mortality rates or reduced viability or fitness, caused by the breeding of closely related individuals, is referred to as inbreeding depression.

Deleterious effects of inbreeding have been documented extensively in humans (e.g., Futuyma 1986:127), laboratory and domestic animals (e.g., Falconer 1960:249), and captive wildlife (e.g., Burgman et al. 1993:220-223). Adverse effects include reduced litter or clutch sizes, higher rates of hatching failure, lower weight of offspring at or soon after birth, reduced survival rates of young and adults, and various physical abnormalities. Inbreeding depression has also been demonstrated in natural (i.e., unrestrained) populations of both plants and animals. For example, among great tits (Parus major), nestling mortality is up to 70% higher among offspring of related, compared to unrelated, parents (Greenwood et al. 1978, van Noordwijk and Scharloo 1981). Among plants that engage in both self-fertilization and out-crossing, the fitness of offspring produced by self-fertilization is often less than half that of offspring produced by out-crossing (Schemske 1983).

Inbreeding, and inbreeding depression, has caused numerous laboratory stocks of animals to die out (Mayr 1974:135). Extinction of wild populations has been documented less often (if ever) because few studies monitoring levels of inbreeding and its deleterious effects have been completed. Nonetheless, the evidence from studies of humans and captive animals provides a firm basis for expecting that inbreeding depression could have serious consequences for population viability.

Inbreeding depression among Kirtland's warblers

Several lines of evidence suggest that Kirtland's warblers are not currently experiencing significant inbreeding depression. Clutch size observed in Carol Bocetti's study was 4.6, about the size recorded in the past from Kirtland's warblers (Walkinshaw 1983) and about the size that would be predicted from considering other species in the same genus. For example, the average size reported by Harrison (1975) for 13 other Dendroica was 4.5. Bocetti (pers. comm.) found low rates of hatching failure and virtually no death of nestlings except from predation. Preliminary estimates of annual survival rates (Kepler pers. comm.) suggest that they are in the range expected based on comparisons with other species. Finally, and perhaps of greatest importance, the warbler population expanded extremely fast during the past several years as new habitat has become available in the Mack Lake burn. The danger posed by inbreeding depression is that it reduces a population's ability to grow or even sustain itself, but in the case of Kirtland's warblers we have empirical evidence that, at present anyway, populations can reach extremely high growth rates when not constrained by habitat.

The absence of inbreeding problems in the Kirtland's warbler population, despite its relatively small population size, is not surprising. For example, Barrowclough (1980) estimated that the effective population sizes of several species of noncolonial birds such as wrens and finches were as low as a few hundred individuals. Empirical evidence suggests that some populations can persist for long periods despite small population sizes. Some island species such as the California Channel Island fox (Gilbert et al. 1990) and the Galapagos hawk (Faaborg, Parker, and Delay unpub.) have high levels of relatedness due to small current or past population size but have persisted for decades or centuries. Populations which were formerly reduced in size and in which relatedness of mates is quite high have also been discovered. The long-finned pilot whale (<u>Glopicephalia melas</u>) is one example of such a species (Amos et al. 1991). Its numbers were sharply reduced by harvest, and relatedness among breeding individuals is high, yet its current population is stable and appears to be viable.

Some populations are more resistant to inbreeding depression than others because of a history of inbreeding. Consistent inbreeding results in exposure of deleterious genes to selection (i.e., homozygous individuals tend to die before reproducing), and this tends to lower the frequency of such genes in the population. For example, many plant species reproduce by self-fertilization as well as cross-fertilization. Some species have populations that vary with respect to the frequency of self-fertilization and cross-fertilization. In one experiment, two groups of offspring were produced by self-fertilization. One group came from a population in which self-fertilization was common. The other group came from a population in which self-fertilization was rare. The offspring from populations where self-fertilization was more common had significantly higher viability (Holtsford and Ellstrand 1990). Thus, effects of a given level of inbreeding may depend on the levels of inbreeding the species has experienced in the past. This in turn may depend on numerous factors including self-fertilization as in the example above, small (or periodically small) population size, or small effective population size caused, for example, by small natal dispersal distances.

Despite the current vitality of the Kirtland's warbler population, some grounds exist for concern over whether genetic problems may develop for the species in the future. The reason is that sharp reductions in population size, such as occurred among Kirtland's warblers during 1960-1985, are often followed by a reduction in genetic variability and a corresponding increase in levels of inbreeding (Hartl and Clark 1985). For example, high levels of inbreeding following severe reductions in population size were reported by Parker et al. (1991) for lions in the Ngorongoro Crater and by Wayne et al. (1991) for gray wolves on Isle Royale. Since fewer individuals are present after the decline in population size, levels of relatedness are higher, or become higher within a few generations. The rate of loss of variability due to drift (chance loss of alleles between generations) is higher in small populations, and the equilibrium level of variability therefore tends to be lower (Futuyma 1986:129-133). Furthermore, past reductions in the size of Kirtland's warblers have often been followed by dramatic expansions in population size (Mayfield 1993), and these may have replenished genetic variability in the warbler. If these increases do not occur in the future (because extremely large fires do not occur), then genetic problems could develop in the species. Thus, even though inbreeding depression does not currently appear to be having severe effects on Kirtland's warblers, it could become a problem in the future.

Predicting whether inbreeding depression actually will occur in the future in Kirtland's warblers is difficult. Franklin (1980), Lande and Barrowclough (1987) and Lande (1988) suggested that inbreeding depression is probably seldom a problem when "effective population size" (see below) exceeds 500. They emphasized that this was intended as a rough guideline and that populations might avoid inbreeding depression, despite much smaller population sizes, through a variety of mechanisms. The proposition that inbreeding depression is seldom a serious problem when effective population size exceeds 500 seems to have withstood both theoretical and empirical analysis.

Effective population size is a concept introduced by Sewall Wright to deal with the problem that real populations differ in important ways from the theoretical populations upon which calculations of genetic drift and inbreeding rates have generally been based. For example, those calculations may assume random mating, non-overlapping generations, a specific distribution in numbers of offspring produced per adult, and so on. Real populations, tend to have some or all of the characteristics just mentioned, however, and this affects rates at which alleles are lost due to genetic drift and rates of inbreeding increase. Wright defined the effective population size of a real population as the number of individuals in an ideal population having the same rate of genetic drift as the real population. Effective population sizes are generally less, and often much less, than real population size (Futuyma 1986:132). For example, Nei and Graur (1984) reviewed studies of 77 species of plants, mammals, fish, reptiles and snails and found that the ratio of effective population size to population size (number of adults present in the population) varied widely but was often less than 1%. Avise et al. (1988) suggested that long term effective sizes of American eels, hardhead catfish, and red-winged blackbirds are less than 1% of current population sizes.

Lande and Barrowclough (1987), Burgman et al. (1993, and references therein) note that effective population size has different meanings in different contexts and provide suggestions for calculating "inbreeding effective population size", the qunatity of relevance here. Sex ratio, lifetime reproductive success - and its variance - for males and females, past population size, generation time, and whether the population can be considered panmictic all affect the calculations, and no single model perfectly fits the Kirtland warbler's natural history. Kirtland's warblers are much better known than most natural populations. Carol Bocetti's recent study of mating behavior and nesting success provides good estimates of the birth rate parameters needed for the calculations. Analysis of the large capture-recapture data set collected during the past several years will provide good estimates of most if not all of the survival and movements parameters needed in the calculations. Once these analyses are completed, deriving an appropriate formula for effective population size will probably be feasible. Thus, while estimation of effective population size for the species at present is hampered by lack of natural history information and a fully appropriate model, both of these problems can probably be solved during the next 1-2 years.

Recommendations

If effective population sizes turn out to be well below 500 (under a specific management plan being proposed), the next step would be to predict whether inbreeding rates are likely to increase in the future, given the species past history and natural history, and if so, how such levels would affect individual and population viability. Finally, the effect of population size on population viability would have to be estimated. Clearly, such estimates would require numerous assumptions, but some insights of value might be possible, and the Recovery Team might feel better ignoring these potential problems in constructing its recovery program if such an effort had been made. This reasoning leads us to make the following recommendations:

1. The simulation model should include genes for each bird because that is easy to do and may help us estimate effective population size.

2. Efforts should be made to estimate effective population size as more information about the species' productivity, survival rates, and dispersal behavior becomes available.

3. If estimated effective population size turns out to be below 500, then the Recovery Team should consider undertaking a more detailed study to predict the likelihood and possible magnitude of future problems due to inbreeding and of how much these possible problems might be affected by the target population size established by the Recovery Team.

4. The computer simulation model we are developing should not include efforts at this time to predict how levels of inbreeding may change in the future or what their effects on individual or population viability might be. Instead, we should make the implicit assumption that inbreeding problems will not reduce viability below current levels.

APPENDIX

Assessing levels of inbreeding

As noted above, populations that remain at the same effective size eventually come into approximate equilibrium between the factors tending to reduce, and to increase, genetic variability. When population size changes, especially if it changes considerably, this equilibrium is upset, the level of variability changes for a time, and then a new equilibrium is established. The change in genetic variability tends to follow an exponential curve, and if the level of variability can be measured at two times, then the parameter for this curve can be estimated and the eventual equilibrium point can be estimated. This is a very general approach to studying genetic variability because it integrates a great many factors affecting the process.

One way to estimate relatedness between individuals of the same species is by using the method known as multilocus minisatellite DNA fingerprinting. DNA fingerprinting uses minisatellite DNA (repetitive units of less than 65 base pairs), from multiple areas of the genome, which is believed to be noncoding and thus not under selective pressure (Jeffreys et al. 1985). An individual's DNA is isolated from a blood sample (easily obtained from birds in the field) and digested (cut into small pieces) with an enzyme. The digested sample of DNA is then drawn through an agarose gel using an electrical current so that the smaller pieces are drawn further through the gel than the larger pieces. The DNA is then transferred from the agarose gel into a nylon membrane using Southern blotting. The nylon membrane is then exposed to radioactive segments of DNA which bind to areas in the individual's DNA which are complementary. A piece of X-ray film is then exposed to the radioactive nylon, resulting in bands, similar to a supermarket bar code, on the X-ray film after it is developed. Because DNA from many individuals can be placed on one agarose gel (up to 26 depending on the gel box size), several individuals' banding patterns can be compared. Bands are determined to be the same if they are the same distance from the top of the gel and of the same intensity. Closely related individuals tend to have similar fragments of DNA and thus tend to share more bands than distantly related individuals. Relatedness of two individuals is thus indicated by their band-sharing score (Piper and Parker Rabenold 1992). The method has also been used as in index to the level of inbreeding in populations. Kuhnlein et al. (1990) showed that band sharing scores were closely related to levels of inbreeding in several strains of chickens. The process of preparing and comparing the bar codes in this manner is known as band-sharing analysis.

Band sharing analysis has proven useful in a number of other conservation issues. For example, Brock and White (1992) compared the endangered Puerto Rican parrot (<u>Amazona vittata</u>) to a less threatened congener, the Hispaniolan parrot (Amazona ventralis) to determine reasons for the lack of success of captive breeding efforts with the Puerto Rican parrot. The captive birds were thought to be unrelated but their band-sharing scores were about the same as the scores from parents and offspring in the Hispaniolan parrot. Furthermore, reproductive success of pairs was closely related to the two birds' relatedness as indicated by their band-sharing scores. The analysis thus revealed that inbreeding was hindering the captive propagation effort.

Determining the band-sharing scores of paired and apparently unrelated Kirtland's warblers, and estimating effective population size for the species, could provide several insights into the likelihood of future genetic problems. Species believed to have large, interbreeding populations tend to have band-sharing scores of 0.25 or less. For example, band-sharing scores were <0.25 for the house sparrow (Passer domesticus, Wetton et al. 1987), 0.25 for the stripe-backed wren (Campylorhynchus nuchalis, Rabenold et al. 1991), and 0.18 for the corn bunting (Miliaria calandra, Hartley et al. 1993). Smaller populations, or populations that have been smaller in the past, tend to have higher scores. For example, banding-sharing scores were 0.44-0.84 for the California Channel Island fox, (Gilbert et al. 1990), 0.70 for the Galapagos hawk (Faaborg, Parker, and Delay unpub.), and 0.56 for the long-finned pilot whale (Amos et al.

1991).

Combining information on current levels of inbreeding from band-sharing analysis with estimates of future levels based on estimates of effective population size would provide useful information on the likelihood of future inbreeding depression. If the band-sharing level was low (e.g., <0.25) and the effective population size was large (e.g., the entire population), then there would be little reason to expect problems with inbreeding depression in the foreseeable future. Conversely, if the band-sharing level was substantially higher and analyses based on effective population size indicated the level might increase substantially during the next decade or two, then monitoring productivity and offspring survival of closely, and distantly, related pairs might be warranted to identify inbreeding depression at an early stage. This latter result would also provide evidence in favor of maintaining a larger, rather than smaller, population. Field techniques and analyses are well-developed for such an effort. Costs would be relatively low (e.g., <$10K), and the results would be of considerable interest in the conservation biology community (because of the large amount known about the warbler population). We can supply additional details about the methods if the Team is interested.

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