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Accounting for Age Structure and Spatial Structure in Eco-Evolutionary Analyses of a Large, Mobile Vertebrate

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Abstract

The idealized concept of a population is integral to ecology, evolutionary biology, and natural resource management. To make analyses tractable, most models adopt simplifying assumptions, which almost inevitably are violated by real species in nature. Here, we focus on both demographic and genetic estimates of effective population size per generation (N_e), the effective number of breeders per year (N_b), and Wright's neighborhood size (N_S) for black bears (*Ursus americanus*) that are continuously distributed in the northern lower peninsula of Michigan, United States. We illustrate practical application of recently developed methods to account for violations of 2 common, simplifying assumptions about populations: 1) reproduction occurs in discrete generations and 2) mating occurs randomly among all individuals. We use a 9-year harvest dataset of >3300 individuals, together with genetic determination of 221 parent–offspring pairs, to estimate male and female vital rates, including age-specific survival, age-specific fecundity, and age-specific variance in fecundity (for which empirical data are rare). We find strong evidence for overdispersed variance in reproductive success of same-age individuals in both sexes, and we show that constraints on litter size have a strong influence on results. We also estimate that another life-history trait that is often ignored (skip breeding by females) has a relatively modest influence, reducing N_b by 9% and increasing N_e by 3%. We conclude that isolation by distance depresses genetic estimates of N_b, which implicitly assume a randomly mating population. Estimated demographic N_S (100, based on parent–offspring dispersal) was similar to genetic N_S (85, based on regression of genetic distance and geographic distance), indicating that the >36000 km^2 study area includes about 4–5 black-bear neighborhoods. Results from this expansive data set provide important insight into effects of violating assumptions when estimating evolutionary parameters for long-lived, free-ranging species. In conjunction with recently developed analytical methodology, the ready availability of nonlethal DNA sampling methods and the ability to rapidly and cheaply survey many thousands of molecular markers should facilitate eco-evolutionary studies like this for many more species in nature.

Subject areas: Conservation genetics and biodiversity; Reproductive strategies and kinship analysis
Keywords: Black bear, demography, dispersal, effective population size, life table, neighborhood size, spatial genetic structure, *Ursus americanus*, Wahlund effect

The idealized concept of a population is integral to both ecology and evolutionary biology (Dobzhansky 1970; Andrewartha and Birch 1984; Krebs 1994; Hedrick 2000; Waples and Gaggiotti 2006). As with most models in evolutionary ecology, this concept adopts simplifying assumptions to make analyses tractable. Almost inevitably, real species in nature violate these assumptions in various ways, and it is important to evaluate the consequences of these departures. Here, we illustrate the practical application of methods to account for violations of 2 common, simplifying assumptions about populations: 1) reproduction occurs in discrete generations and 2) matings occur randomly among all individuals.

Although discrete-generation models provide useful insights, most real species are age structured. Demographic features of age-structured populations have long been studied using components of a standard life table: age-specific survival and fecundity (Cole 1954; Charnov 1993; Caswell 2001). Evolutionary consequences of age structure can be encapsulated in the concept of effective population size (N_e), which is the evolutionary analogue of census size (N). Felsenstein (1971) showed how life-table data could be used to calculate N_e when generations overlap, but his method made simplifying assumptions that limit general applicability. Hill (1972) provided a more general treatment based on the key parameter $V_{k\bullet}$ (lifetime variance in reproductive success among individuals in a newborn cohort), but he did not show how to calculate $V_{k\bullet}$ from vital rates. This latter gap was filled with the AgeNe model (Waples et al. 2011), which incorporates another age-specific vital rate: variance in the reproductive success of individuals of the same age and sex. Other life-history traits such as constraints on litter size and intermittent breeding also can affect N_e and are important to consider as well (Waples and Antao 2014).

Analysis of spatial genetic structuring of species in nature also has a long history, dating to work by Wright (1931; see also Slatkin 1985, Guillot et al. 2009, Milligan et al. 2018). Spatial models consider different levels of demographic/genetic exchange among populations existing in a variety of spatial arrangements, but most retain the assumption that random mating occurs within each subunit. Many species violate this assumption, including those that are continuously distributed across terrestrial or aquatic landscapes. Spatial structure can affect N_e in a variety of ways, depending on factors such as sources of immigrants, rates of extinction and recolonization, and local productivity (Wright 1946; Hedrick and Gilpin 1997; Whitlock and Barton 1997; Ray 2001).

Here, we use an extensive empirical demographic and genetic dataset for black bears (*Ursus americanus*) to illustrate practical application of recently developed methods to explicitly account for both age structure and spatial structure in eco-evolutionary analyses. The dataset includes bears ($N > 3300$; more than 2400 of these with genetic data) harvested over a decade in the northern lower peninsula (NLP) of Michigan, United States (study area > 36 000 km^2). Ages estimated from teeth allowed us to calculate age- and sex-specific survival using life-table analysis (Caughly 1977). Combined with genetic parentage analysis, the age data also allowed us to obtain empirical estimates of both the mean and variance of age-specific fecundity. We adjusted demographic estimates of both N_e (effective size per generation) and N_b (effective number of breeders per year) to account for inherent reproductive constraints (skip breeding and small litter size), and we compared these demographic estimates to genetically derived estimates of effective size that were adjusted for effects of age structure. Finally, from the genetic data and the collection location for each bear, we examined demographic and genetic evidence for isolation by distance and compared demographic and genetic estimates of Wright's (1946) neighborhood size.

It is rare to have such extensive amounts of both genetic and demographic data for a natural population. Joint analyses of these data using recently developed analytic methods provide unparalleled opportunities to infer ecological and micro-evolutionary processes in nature. Black bears are a large, vagile, and charismatic species of management and conservation concern; they also are secretive and difficult to study, so results from this study should be useful throughout their range. These analyses also provide a richer context for interpreting other recent studies of this bear population, which have examined social networks and mating behavior (Moore et al. 2014, 2015), source-sink dynamics (Draheim et al. 2016), and consequences of habitat fragmentation (Draheim et al. 2018). The recently developed analytical methodology illustrated here, combined with the ready availability of nonlethal DNA sampling methods and the ability to survey many thousands of molecular markers rapidly and cheaply, should facilitate eco-evolutionary studies like this for many more species in nature.

Methods

See Table 1 for notation.

Background

The American black bear is the most widely distributed bear species in North America (Lariviere 2001). Breeding occurs in summer, and litters (typically 1–4 young) are born in January or February (Alt 1983; Doan-Crider and Hellgren 1996; Eiler et al. 1989). The NLP in Michigan is a natural unit to study because it is closed to immigration and emigration to the north, east, and west by Lakes Huron and Michigan, and to the south by agricultural lands and urbanization (Figure 1). Using genetic capture-mark-recapture methods, the Michigan Department of Natural Resources estimated the population size of age 1+ black bears in the NLP to be 1882 (95% confidence interval 1389–2551) in 2003 (Dreher et al. 2007); 1675 (966–2385) in 2005 (Etter and Mayhew 2008); and 1500 (1180–1950) in 2009 (MI DNR, unpublished data).

Sample Collection and Preliminary Analyses

Annually, during late September and early October, the state permits hunting of black bears throughout the NLP, and locations of kills are recorded to within 2.6 km^2. In 2002, 2003, and 2006–2010, state biologists collected one tooth (for DNA analysis and ageing) from over 2500 bears harvested in the NLP. Ages were estimated using cementum annuli (Willey 1974). Because bears are born in the winter but hunted in the fall, the last ~9 months is not included in the integer ages derived from teeth. Bears harvested in 2004 and 2005 were not sampled for genetics, but their data were used to estimate age distribution and survival rates.

For the remaining analyses, we used genetic data for 12 microsatellite loci and parentage analysis results reported by Moore et al.

Table 1 Notation and terminology used in the main text

$b_{x(\text{sample})}$	An estimate of relative age-specific fecundity based on a sample of parent–offspring matches
b_x	Mean number of offspring that survive to age 1 produced per time period by an individual of age x; $b_x = b_{x(\text{sample})}$ scaled to produce a stable population
D	Density (number of bears/km^2)
$D_{\text{effective}}$	Effective density (effective number of bears/km^2) = $D \times N_e/N$
F_{IS}	A measure departures from Hardy–Weinberg genotypic proportions
k_i	Number of offspring that survive to age 1 produced in one time period by individual i
$\bar{k}_1 . (\bar{k}_2)$	Mean number of offspring per individual in one time period, before (after) scaling to constant population size; $\bar{k}_2 = b_x$
LD	Linkage disequilibrium; nonrandom associations of alleles at different gene loci
l_x	Cumulative survival through age x
N	Total population size (all bears age 1 and older)
N_1	Total number of yearling offspring produced each year by the population
N_A	Adult population size (assumes all males and 10% of females are mature at age 2, 50% of females are mature at age 3, and the rest mature at age 4).
N_b	Effective number of breeders per year
$\hat{N}_{b(demo)}$, $\hat{N}_{b(geno)}$	Estimates of N_b based on demographic or genetic data
N_e	Effective population size per generation
NS	Wright's neighborhood size, estimated using demographic $(\hat{NS}_{demo})$ or genetic $(\hat{NS}_{geno})$ data
N_x	Number of individuals alive at any given time that are age x
$N^*_{x(\text{sample})}$	Number of parents of age x that could have produced parent–offspring matches
Slope	Slope of the regression of α on ln(z)
s_x	Probability of survival from age x to age $x + 1$
T	Generation length (average age of parents at birth of a cohort); $T = \Sigma x l_x b_x / \Sigma l_x b_x$
V_x	Variance of the k_i among individuals of age x
$V_{k\bullet}$	Lifetime variance in reproductive success among individuals in a single cohort
Wahlund effect	A deficiency of heterozygotes found in a mixture of individuals from genetically divergent populations
x	Age in years
z	Euclidean distance between pairs of individuals
α	Rousset's measure of genetic distance between pairs of individuals
β_{NbNe}	Harmonic mean of demographic N_e and demographic N_b
ϕ_x	Ratio of the variance to the mean reproductive success in one time period of individuals of age x; $\phi_x = V_x/b_x$
σ^2	Variance in mother-offspring dispersal distance, averaged across N-S and E-W directions

(2014). Genetic diversity for black bears in the NLP is generally high and did not vary greatly over years (average heterozygosity = 0.778–0.783; effective number of alleles per locus = 10.22–10.95) or among cohorts. Moore et al. (2014) estimated that 98.5% of mother–offspring dyads were accurately identified. The core dataset for analyses reported here includes genetic data and estimated ages from 2410 bears harvested between 2002 and 2010 (1343 males and 1067 females; Supplementary Table S2).

Estimating Age-Specific Vital Rates

Estimating vital rates involved 3 components: 1) age-specific survival (s_x = probability of surviving from age x to age x+1); 2) age-specific fecundity (b_x = mean number of offspring produced in one season by individuals of age x); and 3) age-specific variance in fecundity (V_x = variance among individuals of age x in the number of offspring produced in one season). V_x and b_x are used to calculate $\phi_x = V_x/b_x$ (the ratio of variance-to-mean reproductive success in one time period of individuals of age x), which is necessary to compute both N_e and N_b. Separate vectors of these age-specific indices were calculated for males and females.

Age-Specific Survival

It is illegal to harvest cubs in their first year, so bears sampled in our study were collected at approximate ages 1.75, 2.75,… In both sexes, the number of age 2.75 bears harvested was anomalously high (Supplementary Table S1, Supplementary Figure S1), which is common for bear populations because sub-adults of both sexes are more vulnerable to harvest (Bunnell and Tait 1985). After log transforming the age-at-death data, we fit an exponential decay model using Poisson regression, which accounts for varying sample sizes with age. The single-survival-rate model produced a poor fit in both sexes, so we estimated separate fits for ages 1–2, 2–3, and 3 and older. More complicated models with survival rates varying annually were not favored by model selection (see Supplementary Table S2 and Detailed Methods in Supporting Information). This produced estimates of s_x = [0.639, 0.559, 0.670] for ages 1–2, 2–3, and 3+ males and s_x = [0.767, 0.619, 0.760] for females in the same age groups (Supplementary Table S4, Figure 2). These estimates account for both natural mortality and harvest mortality. Black bears have been reported to live to 23 years in the wild (Keay 1995), but we set the maximum age at a more realistic 20 years.

Age-Specific Fecundity

Because we do not have data for survival from birth to age 1, we set cumulative survivorship (l_x) to 1.0 at age 1 and assessed fecundity as production of offspring that survived to age 1. Subsequent analyses required estimates of the number of bears alive at any given time, by age and sex (N_x). Although the 3 estimates of abundance (1882, 1675, and 1500 for 2003, 2005, and 2009, respectively) suggest that the population might be declining, these data are not sufficient for a rigorous evaluation of trend. Therefore, we modeled a constant population of N = 1676 bears age 1 and older (the middle of the 3

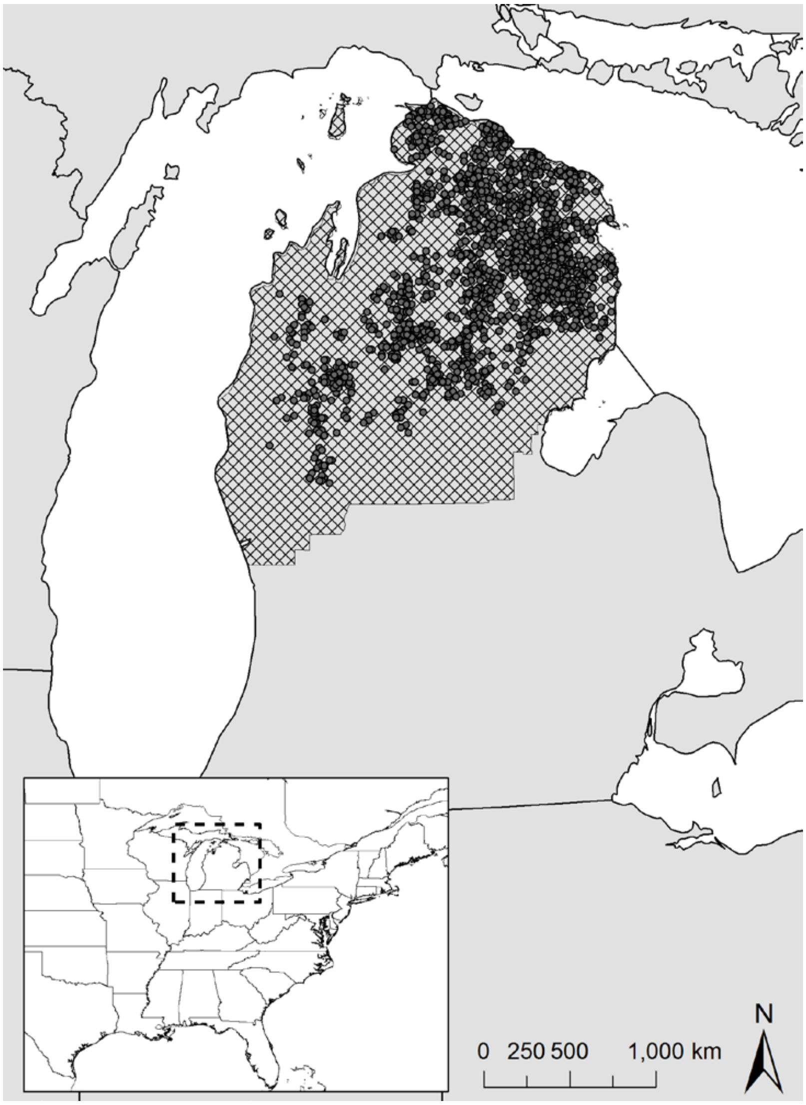

Figure 1. Map of the study area in the northern lower peninsula of Michigan. Cross-hatches define the area included in the mark-recapture estimates of abundance; closed circles are locations of black bears harvested during 2002–2010 that were genetically sampled ($n = 2410$).

estimates), with vital rates as in Supplementary Table S4, and annual recruitment of 520 yearling bears. Assuming an equal sex ratio at birth (consistent with data in Etter et al. 2002), our modeled population included 707 males and 969 females (Supplementary Table S1).

For the 221 bears genetically matched to both parents, the difference between ages of offspring and parents when harvested provided an estimate of ages of the parents when offspring were born. These data in turn were used to estimate mean fecundity and variance in fecundity by age and sex, as described in Detailed Methods and accompanying tables and figures in Supporting Information.

Variance in Age-Specific Fecundity

The final age-specific vital rate to estimate is $\phi_x = V_x / b_x$. The b_x vectors for each sex are given in Supplementary Table S4, so it is only necessary to specify how large V_x is. The default AgeNe assumption is that $\phi_x = 1$ for each age and sex, which assumes Poisson variance in reproductive success ($V_x = b_x$); this is nearly identical to the random binomial variance $V_x = b_x(N_x - 1)/N_x$ unless the number of parents of a given age and sex (N_x) is very small. Under those conditions, each group of individuals of the same age and sex behaves essentially like a mini Wright–Fisher "ideal" population. We are particularly interested in whether variance in reproductive success for each age and sex is overdispersed ($V_x > b_x$) compared with the random expectation, and if so by how much. This is the most complicated part of the statistical analyses and involves several steps, described in Detailed Methods in Supporting Information.

A key step in estimating the ratio of variance-to-mean reproductive success by age and sex is scaling this ratio to the value it would be when age-specific fecundity takes the values required to produce a stable population (b_x). An implicit assumption of the method to rescale the variance-to-mean ratio (see Equation A2 in Detailed Methods in Supporting Information) is that there is no constraint on the number of offspring a parent can produce in one time period. The larger values of $V_{k2}/\bar{k}_2$ shown in Table 2 are only possible if a few individuals each produce large numbers of offspring while most others produce few or none. That might be realistic under Hedgecock's (1994) "sweepstakes reproductive success" hypothesis for marine fish or other species with high batch fecundity, but it is

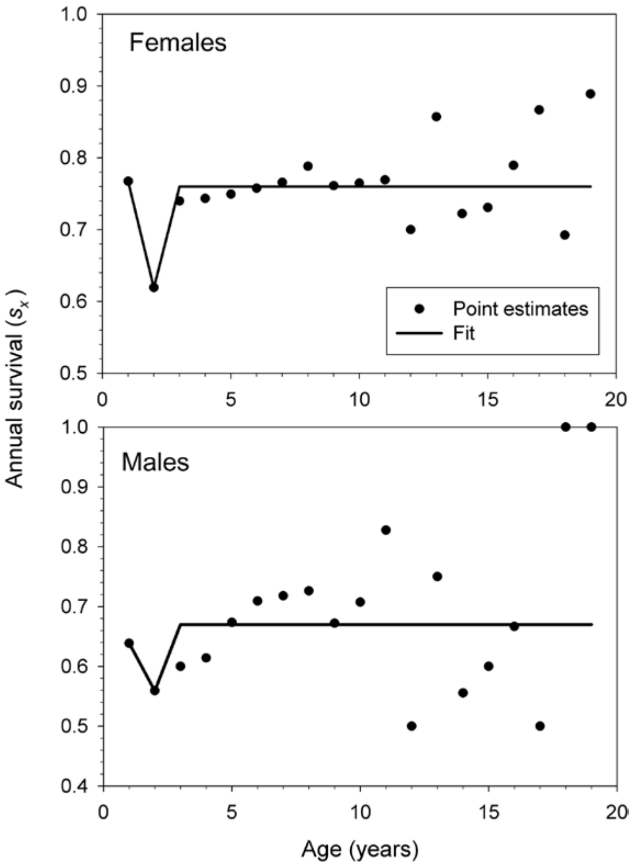

Figure 2. Estimated annual survival rates by age and sex for Michigan black bears. The point estimates were computed from data in Supplementary Table S1 as $s_x = N_{(x+1)Tot}/N_{xTot}$. The fitted estimates (solid lines) represent the best models based on AIC, which have separate estimates for x = 1 and 2 years and a single estimate for ages 3 years and older (see Supplementary Table S2 and Appendix for details).

not feasible for female black bears, which rarely produce more than 1–4 surviving cubs from a single litter (Etter et al. 2002). Therefore, we calculated how large V_{k2} and hence $V_{k2}/\bar{k}_2$ could be within these constraints; this was achieved by assigning k_{max} = 4 offspring to as many females as possible and the remainder to one additional female. Males do not have the same biological constraint on batch fecundity as females, but they still might be limited in the number of female territories they could visit in one breeding season. We set k_{max} = 12 offspring in one time period as the maximum production for males.

Effective Population Size

Demographic Estimates

Following preliminary analyses described above, we used program AgeNe (Waples et al. 2011) to estimate demographic N_b and N_e. AgeNe uses the birth-pulse model of reproduction, in which all births are assumed to occur at integer parental ages (Caswell 2001). Individuals that die between ages x and x+1 are assumed to have reproduced at age x before dying. AgeNe calculates N_e by grouping individuals from a single cohort by age at death (see Detailed Methods in Supplementary Information). This allows one to calculate lifetime variance in reproductive success across all members of a cohort ($V_{k\bullet}$), which is then used in Hill's (1972) formula to calculate N_e per generation:

$$N_e = \frac{4N_1T}{V_{k\bullet}+2}. \qquad (1)$$

In Equation 1, which assumes stable age structure and constant population size, N_1 is the number of offspring in each cohort and $T = \Sigma x l_x b_x / \Sigma l_x b_x$ is generation length.

The effective number of breeders per year (N_b) is calculated from the standard formula for inbreeding effective size with separate sexes (e.g., Crow and Denniston 1988):

$$N_b = \frac{\bar{k}N-2}{\bar{k}-1+V_k/\bar{k}}, \qquad (2)$$

where $\bar{k}$ and V_k are the mean and variance in per-capita number of offspring produced in 1 year, calculated across all N adults in the population.

One final adjustment to the demographic estimates of effective size is necessary, as female black bears typically skip reproduction for 1 or more years after giving birth (Eiler et al. 1989). This limits the pool of female breeders available in a given year and reduces both female N_b and overall N_b. Conversely, this also insures that different females reproduce in different years, which reduces lifetime variance in reproductive success and hence increases N_e per generation (Waples and Antao 2014).

Available data from Michigan (Boersen, unpublished data) suggest that females that gave birth in year t have only a 3.5% chance of reproducing in year t+1 and a 94% probability of reproducing in year t+2; previous reproduction is not thought to affect subsequent births 3+ years later. Based on these probabilities, we used the method of Waples and Antao (2014) to calculate the actual number of female bears that reproduce each year and, given the above rules, the number that are unavailable because of recent births. A key step in this analysis is calculating the fraction of females that, just by chance, would not produce any offspring in a given year, even though they were available to breed. The R scripts provided by Waples and Antao (2014) assumed a random distribution of offspring number, which would apply to all females breeding in one year only if fecundity was constant with age and ϕ was fixed at the binomial variance $(N_x - 1)/N_x$. Neither of these is true for female black bears, so we modified the code to allow overdispersed variance in reproductive success. We used the overall female $V_k/\bar{k}$ from AgeNe output (1.442/0.536 = 2.69; Table 3), which applies to all females that potentially mate in 1 year and accounts for both age-specific differences in fecundity and overdispersed variance among same-age individuals.

Genetic Estimates

Estimated ages were used to group individuals into a series of cohorts of bears born in the same year. Samples from each cohort were used to estimate N_b using the linkage disequilibrium (LD) method implemented in program LDNe (Waples and Do 2008). To minimize bias, we excluded rare alleles with frequencies <0.02. To account for the effects of age structure on our genetic estimates of N_b, we used the formula in Table 3 of Waples et al. (2014):

$$\hat{N}_{b(Adj)} = \frac{\hat{N}_b}{1.26 - 0.323*(N_b/N_e)}, \qquad (3)$$

where $\hat{N}_b$ is the raw estimate from LDNe, N_b/N_e is the demographic estimate of the ratio of annual and generational effective size (from AgeNe as described above), and $\hat{N}_{b(Adj)}$ is the adjusted genetic estimate. This adjustment reflects the fact that single-cohort estimates are affected by residual LD from previous generations, which is a function of N_e per generation.

Isolation by Distance

Demographic Estimates

Demographic estimates of neighborhood size (*NS*) used the relationship described by Wright (1946):

$$NS_{\text{demo}} = 4\pi\sigma^2 D_{\text{effective}}, \qquad (4)$$

where $D_{\text{effective}}$ is the effective density (effective number of individuals per unit area), π is pi, and σ^2 (the variance of the signed parent–offspring distance along one axis, δ) is a measure of dispersal. The raw measure of density (*D* = number of bears age 1 and older per km^2) was multiplied by the estimated N_e/N ratio (from AgeNe as described above) to estimate $D_{\text{effective}}$.

In Wright's model, δ is the difference in location (along one axis) between the birth of the parent and the birth of its offspring. We lack birth locations, so instead we used the difference in location of death of the mother and the death of her offspring. We only used mother–offspring data for these analyses because, unlike males, females generally establish a permanent home range near the age at maturity. For each mother–offspring pair, we calculated the signed, axial dispersal distance (km) in 2 directions: δ_{ew} for east–west and δ_{ns} for north–south. If dispersal were perfectly symmetrical north–south and east–west, as assumed by Wright (1946), means across all mother–offspring pairs ($\bar{\delta}_{ew}$ and $\bar{\delta}_{ns}$) should be 0. We calculated the variance of δ_{ew} and δ_{ns} and took the mean as our estimate of σ^2.

Table 2. Distribution of numbers of offspring (*k*) produced by male and female black bears at specified ages (from Moore et al. 2014)

		k													$V_{k2} / \bar{k}_2 = \phi_x$	
Age	Offspring	1	2	3	4	5	$\sum k_i$	$\sum (k_i)^2$	$N^*_{x(\text{sample})}$	$\bar{k}_1$	V_{k1}	$V_{k1} / \bar{k}_1$	$\bar{k}_2$	$\bar{k}_2 / \bar{k}_1$	Raw	Modeled
Males																
2	36.5	36.5					36.5	36.5	1343	0.027	0.026	0.974	0.544	20.0	1.00	1.098
3	25.5	23.5	1				25.5	27.5	849	0.030	0.032	1.050	0.601	20.0	1.99	1.934
4	16	14	1				16	18	534	0.030	0.033	1.097	0.600	20.0	2.94	2.770
5	22	18	2				22	26	357	0.062	0.069	1.123	1.232	20.0	3.47	3.606
6	29	18	4	1			29	43	239	0.121	0.166	1.367	2.425	20.0	8.35	10.807[a]
7	25	10	5			1	25	55	160	0.156	0.321	2.057	3.124	20.0	22.15	10.807
8	21	6	6	1			21	39	107	0.196	0.329	1.677	3.924	20.0	14.54	10.807
9	17	8	3	1			17	29	72	0.238	0.354	1.489	4.754	20.0	10.79	10.807
10	18	11	2	1			18	28	48	0.377	0.454	1.204	7.544	20.0	5.08	10.807
Females																
3	23	18	1	1			23	31	562	0.041	0.054	1.309	0.422	10.3	4.19	3.210[b]
4	34	26	4				34	42	426	0.080	0.092	1.158	0.823	10.3	2.63	3.210
5	25	16	3	1			25	37	323	0.077	0.109	1.407	0.799	10.3	5.20	3.210
6	41	28	5	1			41	57	245	0.168	0.206	1.228	1.730	10.3	3.35	3.210
7	16	10	3				16	22	185	0.087	0.112	1.295	0.893	10.3	4.05	3.210
8	18	16	1				18	20	140	0.129	0.128	0.989	1.331	10.3	0.89	3.210
9	18	12	3				18	24	105	0.171	0.201	1.173	1.768	10.3	2.79	3.210
10	13	5	4				13	21	79	0.165	0.242	1.469	1.701	10.3	5.84	3.210

$N^*_{x(\text{sample})}$ is the number of parents of each age and sex whose offspring might have appeared in the sample (from Table 1 and Supplementary Table S3). $\bar{k}_1 = \Sigma k_i / N^*_{x(\text{sample})}$ is the mean *k* for the sample and V_{k1} is the sample variance of *k* among parents (calculated from Equation 1, with $n = N^*_{x(\text{sample})}$). $\bar{k}_2$ (b_x from Supplementary Table S4) is the mean *k* for that age and sex that produces a stable population. Raw $V_{k2} / \bar{k}_2 = \phi_x$ is the $V_{k1} / \bar{k}_1$ ratio scaled to $\bar{k}_2$ according to Equation 3. Modeled $V_{k2} / \bar{k}_2$ is described in the text. The ratio $\bar{k}_2 / \bar{k}_1$ (20.0 for males and 10.3 for females) was the scaling factor used in Equation 3.

[a] geometric mean for raw male $V_{k2} / \bar{k}_2$ ages 6–10

[b] geometric mean for raw female $V_{k2} / \bar{k}_2$ ages 3–10

Table 3. Output from program AgeNe (Waples et al. 2011) using estimated vital rates described in previous sections and "middle" values of ϕ_x from Supplementary Table S5

	Per year				Lifetime, for a single cohort				
	N	$\bar{k}_2$	V_k	N_b	N	T	$\bar{k}$	$V_{k\bullet}$	N_e
Female	969	0.536	1.442	233	260	7.10	2	19.43	345
Male	707	0.735	5.152	77	260	5.96	2	35.53	165
Total	1676	0.620	3.016	232	520	6.53	2	27.48	461

Values per year were calculated over all individuals in the population; lifetime values were calculated for all N_1 = 520 individuals in a cohort of age-1 black bears. $T = \Sigma x l_x b_x / \Sigma l_x b_x$ is generation length, N_e is the effective population size per generation, N_b is the effective number of breeding adults per year, and $V_{k\bullet}$ is the lifetime variance in reproductive success across all members of a cohort. This analysis does not account for skip breeding.

Genetic Estimates

Genetic estimates of *NS* followed Rousset (2000). We examined the relationship between genetic distance for pairs of individuals *i* and *j* (α_{ij}) and Euclidean distance that separates them (z_{ij}). For 2-dimensional isolation by distance that conforms to Wright's model, the inverse of the slope of the regression of α_{ij} on $\ln(z_{ij}) = 4\pi\sigma^2 D_{\text{effective}}$, so a genetic estimate of neighborhood size is:

$$NS_{\text{geno}} = 1 / Slope. \tag{5}$$

The program SPAGeDi Version 1.3 (Hardy and Vekemans, 2002) was used to calculate α and z.

To provide another perspective on the spatial autocorrelation of genotypes, individual-based autocorrelation coefficients (r; range −1 to +1) characterizing inter-individual relatedness as a function of geographic distance were estimated from pairwise genetic distances (Smouse and Peakall 1999) and pairwise z values among all individuals using GenAlEx v6 (Peakall and Smouse 2006). The null hypothesis was $r = 0$, indicating no correlation between genetic and geographic distance, and significance of r was determined using permutations ($N = 999$) to estimate 95% confidence intervals around zero.

Effects of the spatial scale of sampling were evaluated using 2 genetic indices—$\hat{N}_e$ from LDNe and F_{IS} (Weir and Cockerham 1984, as implemented in FSTAT, Goudet 2001)—in 10 replicate sampling areas randomly placed across the study area. F_{IS} measures departures from Hardy–Weinberg proportions, with positive values indicating heterozygote deficiencies and negative values indicating excesses. Four geographic scales of sampling were considered: 1) circles of 1400 km^2; 2) circles of 3500 km^2; 3) 2 halves of the study area (east and west); and 4) the entire study area. For these analyses, the total area subject to sampling was defined by a minimum complex polygon (area ~27 000 km^2) that includes all of the bears from which genetic data were collected (filled circles in Figure 1).

Results

Accounting for Age Structure

Population Demography

Male black bears experienced higher mortality than females at all ages, so, even if sex ratio is equal at birth, the overall population is expected to be approximately 58% female (969/1676, Supplementary Table S1). At age 20, 0.5% of females that reached age 1 are expected to still be alive, whereas <0.1% of males would survive that long. Age 20 is a somewhat arbitrary point to truncate the life table, but with such small l_x values at that age, extending the lifespan would have little effect on downstream analyses. Estimated age-specific fecundity (b_x), smoothed and scaled to the production of offspring that survived to age 1, rose slowly in females until age 6, after which the point estimate of b_x based on genetic pedigree analysis remained relatively constant (Supplementary Table S4B; Figure 3). The jump in estimated fecundity at age 6 followed by a dip at age 7 (Figure 3) could reflect combined effects of variable age at maturity and skip breeding. If bears that mature early at ages 2–3 require multiple years to recover before reproducing again, while bears that mature at age 4 typically need only 1 year, a large fraction of the adult females would be reproducing for a second time at age 6, after which most would have to skip at least 1 year before having another litter.

In males, estimated b_x was <1 until age 5, after which point fecundity rose sharply and steadily to reach 7.5 at age 10. The small numbers of bears (especially males) in older age classes complicated age-specific estimates beyond age 10; however, available data suggest that reproductive success declines sharply in older males, whereas females showed little evidence of reproductive senescence. Generation length for this population was estimated to be 6.53 years (5.96 males; 7.10 females).

The 3358 harvested bears in our dataset collected over 9 years (Supplementary Table S1) translate as an annual harvest of 207 males and 166 females, which (based on modeled constant abundance of 969 females and 707 males of age 1 and older) represent estimated annual harvest rates of 29% for males and 17% for females. The modeled constant population has 520 births and deaths each year (260 of each sex), so overall annual mortality rates are 260/707 = 0.368 for males and 260/969 = 0.268 for females. Thus, we estimate that harvest comprises 0.29/0.368 = 80% of total annual mortality for males and 0.17/0.268 = 64% for females.

Variance in Reproductive Success

Raw estimated $V_{k1} / \bar{k}_1$ for females was significantly higher than the random expectation for all ages except age 8 years; in males, $V_{k1} / \bar{k}_1$ was compatible with random reproductive success for ages 2–5 and 10 years but significantly overdispersed for ages 6–9 years (Supplementary Figure S8). Rescaling the $V_{k1} / \bar{k}_1$ ratio to the absolute mean fecundity (b_x) is required to calculate age-specific ϕ_x and hence effective population size. Doing this substantially increased the raw $V_{k2} / \bar{k}_2$ values, to as high as 23.2 for males and 6.2 for females (Table 2). This adjustment made a substantial difference, because the adjustment factor ($\bar{k}_2 / \bar{k}_1$; see Equation A2) was 20 in males and 10.3 in females.

To illustrate these calculations, 29 of the 221 matched offspring were assigned to age-6 male bears (Table 2). Of those male parents, 18 produced exactly 1 offspring that appeared in our sample, 4 produced 2, and 1 produced 3. We estimated the total potential sample size as $N_{x(\text{sample})}$ = 239 age-6 males in a stable population, so $\bar{k}_1$ = 29/239 = 0.121. Using n = 239 in Equation A1 along with the values for Σk and Σk^2 from Table 2, V_{k1} = 0.166, so $V_{k1} / \bar{k}_1$ = 1.37, which is significantly greater than 0 (Supplementary Figure S8), and indicates greater than random variation in reproductive success.

Figure 3. Standardized age-specific fecundity estimated for Michigan black bears. These standardized values adjust the $b_{x(\text{sample})}$ values from Supplementary Figure S7 to produce a population of constant size and are expressed in terms of offspring that survive to age 1 year. Values for ages 11–20 years use combined data for all ages >10 years.

However, this underestimates the degree of overdispersion: the stable-population mean fecundity ($\bar{k}_2 = b_6 = 2.426$) is 20 times as large as $\bar{k}_1$, so the adjusted estimate of ϕ_6 for males is also much larger ($V_{k2} / \bar{k}_2 = 8.35$).

To reduce the effects of random variation in estimated $V_{k2} / \bar{k}_2$ caused by small sample sizes of offspring genetically assigned parents for each age and sex, we considered ways to model "smoothed" values. There is no evidence of a linear trend with age in female $V_{k2} / \bar{k}_2$ ($P > 0.5$), so we modeled $V_{k2} / \bar{k}_2$ in females as a single value (3.21) equal to the geometric mean of the values for ages 3–10 years. Males showed a positive correlation of raw $V_{k2} / \bar{k}_2$ with age ($r = 0.56$), but the pattern was uneven and not statistically significant ($P > 0.1$). However, male $V_{k2} / \bar{k}_2$ increased steadily for ages 2–5 years ($r = 0.99$; $P < 0.05$), so we modeled $V_{k2} / \bar{k}_2$ in males as a 2-step process: a linear increase through age 5 years, and a single value for ages 6–10 years equal to the geometric mean for those ages (10.807) (Figure S9).

The final step in assessing age-specific variance in reproductive success considered biological constraints on the number of offspring that could be produced in a single year. The scaled $V_{k2} / \bar{k}_2$ values were based on an implicit assumption that a given parent can produce an arbitrarily large number of offspring per time period. Constraining V_k in females to the maximum it could be if litter size cannot exceed 4 cubs showed that maximum plausible values of $V_k / \bar{k}$ for females were lower than the single modeled value (3.21) for some ages. For example, the maximum possible value of $V_k / \bar{k}$ was <3 for 6 year-old and 8–10 year-old females. Because this 4-cub maximum scenario was extreme in allocating the maximum number of offspring to as many females as possible and zero offspring to all the rest, we used the results for $k_{max} = 4$ as our "high" estimate of $V_k / \bar{k}$ for females, and we used the raw (unadjusted) $V_{k1} / \bar{k}_1$ values as our "low" estimate of female $V_k / \bar{k}$. For the "middle" estimate of female ϕ_x, we used the mean of the "high" and "low" values, and these constrained estimates of female ϕ_x fell between 1.74 and 2.41 (Supplementary Table S5).

Noting that the estimates of mean offspring number were as high as 7.5 for age-10 males (Supplementary Table S4A), we calculated maximum possible values for V_k and $\bar{k}$ under the assumption that males could father no more than $k_{max} = 12$ offspring in a single year. This reduced ϕ_x for ages 6 years and above (Supplementary Table S5). In both sexes, because sufficient data were not available to estimate $V_k / \bar{k}$ for bears over 10 years of age, we used the geometric mean of the estimates for ages 6 to 10 years for older bears. High, low, and middle estimates of ϕ_x for males and females are shown in Supplementary Figure S10.

Demographic Estimates of Effective Size

Using the "middle" estimates of ϕ_x and other vital rates from Supplementary Table S4 in program AgeNe, estimated N_e was 461 and estimated N_b was 232, for an estimated ratio N_b/N_e of 0.5 (Table 3). Female N_b and N_e are both much higher than the corresponding values for males, but the disparity is greater for annual effective size (female N_b is 3× male N_b) than for generational effective size (female/male $N_e = 2.1$). Table 4 shows how N_b, N_e, and effective size ratios responded to different ways of modeling age-specific reproductive success. Under the "high" and "low" assumptions for ϕ_x (Scenarios III and IV), the N_b/N_e ratios were 0.43 and 0.89, respectively. This occurred because higher values of ϕ_x reduce N_b more than N_e, so the ratio goes down. These results also illustrate the vital importance of obtaining empirical estimates of age-specific variance in reproductive success: using the default AgeNe assumption that $\phi_x = 1$ for every age in both sexes (Scenario I), the estimated ratio N_b/N_e was 1.02—just over twice as large as the point estimate based on empirical data for Michigan bears. As demonstrated below, this has important consequences for adjustments to genetic estimates of effective size to account for age structure.

These estimates account for constraints on litter size and mate availability, but one final adjustment is necessary to account for skip breeding by females. Using the method of Waples and Antao (2014) as described in Methods to modify Scenario II in Table 4, we estimate that in any given year skip-breeding constraints will reduce female N_b by 28% (from 233 to 168), and this will reduce overall N_b by about 9% (from 232 to 211). Because overall N_b is strongly influenced by the low male N_b (77), reducing the higher female N_b does not have a large effect on overall N_b. Based on modeled results for the grizzly bear from Waples and Antao (2014), we estimated that skip breeding will increase N_e by about 3% in black bears, from 461 to 475. This leads to a comprehensive estimate of $N_b/N_e = 211/475 = 0.44$ (Scenario V, Table 4). In a final variation, we modified Scenario III (high estimates of ϕ_x) for skip breeding; results are shown as Scenario VI. Under this scenario, N_b/N_e was reduced to 0.38.

Genetic estimates of effective size.—The quality and breadth of data available for Michigan black bears provides an opportunity to illustrate adjustments to parameter estimates in cases where underlying assumptions of simple models are clearly violated. To simplify the analyses and to allow a direct estimate of the effective number of bears reproducing each year (N_b), we first used the age data to group bears into cohorts based on year of birth. The result (Table 5) was 2273 bears sorted into 12 cohorts (1998–2009), with sample sizes ranging from 68 to 342 (harmonic mean = 146). Raw estimates of the effective number of breeders producing each cohort ranged from $\hat{N}_b = 149$ to 264 (harmonic mean 195). Adjustment to raw $\hat{N}_b$ to account for effects of age structure

Table 4. Demographic estimates of N_e and N_b, and several effective size ratios for 6 scenarios that make different assumptions about age-specific reproductive success

	Scenario					
	I	II	III	IV	V	VI
N	1676	1676	1676	1676	1676	1676
N_A	916	916	916	916	916	916
N_e	562	461	427	549	475	440
N_b	564	232	183	490	211	166
N_b/N_e	1.00	0.50	0.43	0.89	0.44	0.38
N_e/N	0.34	0.28	0.25	0.33	0.28	0.26
N_e/N_A	0.61	0.50	0.47	0.60	0.52	0.48
N_b/N	0.34	0.14	0.11	0.29	0.13	0.10
N_b/N_A	0.62	0.25	0.20	0.53	0.23	0.18

N is the total number of black bears age 1 and older; N_A = the number of mature bears (assumes 10% of females and all males are mature at age 2 years, 50% of females are mature at age 3 years, and the remainder mature at age 4 years).

Scenarios:

I Assumes $\phi_x = 1$ for all ages and sexes

II Uses "middle" estimates of ϕ_x for each age and sex (from Supplementary Table S5)

III Uses "high" estimates of ϕ_x

IV Uses "low" estimates of ϕ_x

V Adjusts Scenario II for skip breeding

VI Adjusts Scenario III for skip breeding

Table 5. Adjustments to genetic estimates of N_b to account for age structure

Cohort	S	$\hat{N}_b$	$\hat{N}_{b(adj)}$	$\hat{N}_{b(adj)}/\hat{N}_{b(demo)}$	β_{NbNe}	$\hat{N}_b/\beta_{NbNe}$
1998	68	204 (131–420)	183 (117–376)	0.87	292	0.70
1999	84	264 (167–565)	236 (150–506)	1.12	292	0.90
2000	139	221 (171–303)	198 (153–271)	0.94	292	0.76
2001	226	196 (166–235)	175 (149–210)	0.83	292	0.67
2002	223	191 (162–228)	171 (145–204)	0.81	292	0.65
2003	136	260 (194–279)	233 (174–250)	1.10	292	0.89
2004	247	233 (195–285)	209 (175–255)	0.99	292	0.80
2005	274	149 (131–171)	134 (118–153)	0.63	292	0.51
2006	342	178 (158–203)	160 (141–181)	0.76	292	0.61
2007	251	167 (145–195)	150 (130–175)	0.71	292	0.57
2008	205	205 (171–251)	183 (153–225)	0.87	292	0.70
2009	78	151 (110–233)	136 (98–209)	0.64	292	0.52
Mean[a]	146	195	175	0.84		0.68

This example uses results from Scenario V in Table 4, for which total abundance = 1676 age-1+ bears, demographic $N_e = 475$, and demographic N_b ($\hat{N}_{b(demo)}$) = 211. S = number of individuals sampled from each cohort; $\hat{N}_b$ = raw genetic estimate of N_b (from program LDNe, with 95% confidence intervals in parentheses); $\hat{N}_{b(adj)} = \hat{N}_b$ adjusted for age structure using Equation 6; $\hat{N}_{b(adj)}/\hat{N}_{b(demo)}$ = ratio of adjusted genetic and demographic estimates of N_b; β_{NbNe} = harmonic mean of demographic N_e and demographic N_b; $\hat{N}_b/\beta_{NbNe}$ = ratio of the raw genetic estimate of N_b to harmonic mean of demographic N_e and N_b. See Supplementary Table S6 for similar results for Scenario VI.

[a] Harmonic mean for S, $\hat{N}_b$, and $\hat{N}_{b(adj)}$; geometric mean for the ratios $\hat{N}_{b(adj)}/\hat{N}_{b(demo)}$ and $\hat{N}_b/\beta_{NbNe}$.

depends on the true ratio N_b/N_e (Equation 3). We applied this correction using demographic estimates of N_b and N_e for the 2 scenarios in Table 4 that accounted for skip breeding (Scenarios V and VI, which used the "middle" and "high" estimates of ϕ_x, respectively). For Scenario V, the adjusted genetic estimates ($\hat{N}_{b(adj)}$) were reduced by about 10% (harmonic mean $\hat{N}_{b(adj)}$ = 175 vs. 195 for raw $\hat{N}_b$; Table 5). These adjusted genetic estimates can be compared to the single demographic estimate of N_b, which was 211 for Scenario V. The yearly ratios of $\hat{N}_{b(adj)}/\hat{N}_{b(demo)}$ ranged from 0.63 to 1.12 (geometric mean 0.84), indicating that the adjusted genetic estimates were on average 16% lower than the demographic estimate. Under Scenario VI, the genetic and demographic estimates of N_b are in better agreement (geometric mean $\hat{N}_{b(adj)}/\hat{N}_{b(demo)}$ = 1.05; range = 0.79–1.40; Supplementary Table S6).

Across the 12 cohorts, we found a negative correlation between year and $\hat{N}_{b(adj)}$, which was not quite statistically significant at the 0.05 level ($r = 0.574$ vs. a critical value of 0.576 for a 2-tailed test of no relationship). Thus, there is a suggestion that the effective number of breeders per year might have declined over the study period.

It is of interest to compare the raw genetic estimates of N_b with the harmonic mean of demographic N_e and N_b (β_{NbNe}), which is the quantity they in theory are estimating (Waples et al. 2014). For Scenario V, $\hat{N}_b/\beta_{NbNe}$ ranged from 0.51 to 0.90, with a geometric mean of 0.68 (Table 5). Thus, on average, the raw genetic estimates were about two-thirds of the harmonic mean of N_e and N_b. Under Scenario VI, β_{NbNe} was lower, which raised the geometric mean $\hat{N}_b/\beta_{NbNe}$ to 0.82 (Supplementary Table S6).

Accounting for Spatial Structure

Demographic Data

Moore et al. (2014, 2015) presented evidence for greater dispersal distances of male than female black bears in the NLP, so we estimated demographic parameters and neighborhood size (NS_{demo}) separately by sex. If mother–offspring dispersal distance also varied with age in adults, then the assumption that the location of death was a representative of the mean location of all offspring produced would be violated. We found significantly higher dispersal distances in males than females for ages 1–5 years and also for age 8 years and above, although the older ages produced few data points (Figure 4). This indicates that black bear male neighborhoods are substantially larger than those for females. There was no apparent trend of mother–daughter dispersal with age. We found a complex pattern for mother–son: sharp increases from ages 1–3, followed by a sharp decline through age 7. Information for older males was sparse. Based on these results, we did not attempt any quantitative adjustments to account for age-related changes in dispersal.

The modeled population included N = 1676 age 1+ bears (707 males and 969 females). The area relevant to the mark-recapture estimates of abundance covered 36 848 km^2 (Dreher et al. 2007), so total density was D = 0.0455 bears/km^2, with sex-specific densities of 0.0192/km^2 for males and 0.0263/km^2 for females. Adjusting for the N_e/N ratio using the best point estimate of N_e/N = 0.28 (Scenario V, Table 4) brought point estimates of effective density to $D_{\text{effective}}$ = 0.0054 males/km^2 and 0.0074 females/km^2 (Table 6A). We also calculated effective density using the higher and lower estimates of abundance (N = 1882 and 1500, respectively).

For both sexes the mean axial dispersal distance (δ) for mother–offspring dyads was close to zero km in both east-–west and north–south directions (Table 6A), as would be expected if dispersal were symmetrical (an assumption of Wright's neighborhood-size model). Variances in axial dispersal distance (σ^2) were also roughly comparable in the 2 directions, but mean σ^2 across both directions was almost 4 times as high for males (1401 km^2) as for females (365 km^2). Combining these estimates of σ^2 and $D_{\text{effective}}$ in Equation 4 and assuming a population of 1676 bears, estimated demographic neighborhood sizes are $NS_{\text{demo,m}}$ = 95 for males and $NS_{\text{demo,f}}$ = 34 for females. Using a variation of Wright's (1938) formula for adjusting effective size for unequal sex ratio ($N_e = 4MF/(M+F)$, where M and F are effective numbers of males and females) produces the overall estimate of NS_{demo} = 4*95*34/(95 + 34) = 100. Using the high and low estimates of abundance, the estimates of NS_{demo} were 112 and 85, respectively (Table 6A).

Genetic Data

Before estimating genetic neighborhood size, we evaluated the assumption that the relationship between Rousset's (2000) pairwise distance α and ln(*z*) was linear, as assumed in Wright's model and in Equation 5. When individuals were grouped into bins defined by dispersal distance, the overall data supported this assumption, as the proportion of variance explained by the linear fit was $r^2 = 0.84$ (Figure 5). Separate analyses for 3 time periods (2002–03, 2006–07, and 2009–10) showed somewhat more variability and possible edge effects (Supplementary Figure S11). To calculate the slope of this relationship to estimate NS_{genetic} (Equation 5), we used all data points for comparisons involving distances >σ, as recommended by Rousset (2000). Mean σ² across males and females was 883, leading to σ = 29.7, so we only used comparisons of individuals separated by at least 30 km. We calculated the slope separately for individuals collected in the 3 time periods. Resulting slopes ranged from 0.0092 to 0.0148, which produced estimates of genetic neighborhood size of $NS_{\text{genetic}} = 109$, 89, and 68, respectively (Table 6B). Using the average of the 3 slopes, the overall estimate was $NS_{\text{genetic}} = 85$. We found additional support for genetic isolation by distance in the plot of individual spatial autocorrelation coefficients (*r*) and geographic distance (Supplementary Figure S12). Genetic correlations among pairs of individuals were significantly positive up to a range of about 40 km, and this pattern was consistent across time periods.

Figure 4. Estimated mother–offspring dispersal distance for Michigan black bears, by sex and age. Error bars represent 2 standard deviations around the mean; symbols without error bars (age ≥8 years) are for single observations. Ages for males and females are jittered to avoid overlap.

How large is a "neighborhood" of Michigan black bears? The area encompassing one genetic neighborhood is $NS/D_{\text{effective}}$. Using the middle point estimate of $NS_{demo} = 100$, this area is 100/0.0127 = 7874 km²; using the overall estimate of $NS_{\text{genetic}} = 85$, this area is 100/0.0117 = 8547 km². Thus, we estimate that the study area (36 848 km²) includes between 4 and 5 black bear neighborhoods, sensu Wright.

Consistent with the above results, we found that as the geographic area of sampling expanded, F_{IS} became more positive, indicating larger heterozygote deficiencies (Figure 6 top). This presumably reflected an increasingly strong Wahlund effect as larger sampling areas combined individuals with more divergent genetic backgrounds. A strong Wahlund effect was also apparent in the pattern of change in $\hat{N}_e$ with spatial scale of sampling. Samples encompassing larger geographic areas also produced larger estimates of effective size, but $\hat{N}_e$ increased at a much slower rate

Table 6. Estimating neighborhood size

A.

Abundance estimate	Sex	N	D	$D_{\text{effective}}$	Mean (variance, σ²) in axial dispersal			$\widehat{NS}_{demo}$
					East–west	North–south	Overall σ²	
Middle 2005	Male	707	0.019	0.0054	1.16 (1231)	−2.62 (1570)	1401	95
	Female	969	0.026	0.0074	0.42 (427)	1.91 (303)	365	34
	Total	1676	0.045	0.0127				100
Low 2009	Male	633	0.017	0.0048	1.16 (1231)	−2.62 (1570)	1401	85
	Female	867	0.024	0.0066	0.42 (427)	1.91 (303)	365	30
	Total	1500	0.041	0.0114				89
High 2003	Male	794	0.022	0.0060	1.16 (1231)	−2.62 (1570)	1401	106
	Female	1088	0.030	0.0083	0.42 (427)	1.91 (303)	365	38
	Total	1882	0.051	0.0143				112

B.

Time period	n	slope	$\widehat{NS}_{geno}$
2002–2003	635	0.0092	109
2006–2007	719	0.0112	89
2009–2010	594	0.0148	68
	Mean	0.0117	85

A: demographic estimates ($\widehat{NS}_{demo}$). Results are shown for 3 estimates of abundance; numbers of males and females assume the same sex ratio as shown in Table 3. Total density (*D*, bears/km²) uses the area for the capture-mark-recapture estimates (36 848 km²). Effective density ($D_{\text{effective}}$, effective number of bears/km²) = $D*N_e/N = 0.28D$, based on the best point estimate of N_e/N (Scenario V from Table 4). σ² is the variance in axial mother–offspring dispersal distance in km. Overall σ² is the average across east–west and north–south directions. $\widehat{NS}_{demo}$ for each sex was calculated from Equation 7, and overall neighborhood size was calculated as $\widehat{NS}_{demo} = 4NS_{\text{male}}NS_{\text{female}}/(NS_{\text{male}} + NS_{\text{female}})$ after Wright (1938). B: genetic estimates ($\widehat{NS}_{geno}$). Separate estimates were computed for 3 time periods, using *n* = 594–719 bears. "slope" is the slope of the regression of Roussset's α on ln(*z*), where *z* is Euclidean distance between pairs of individuals. $\widehat{NS}_{geno} = 1/\text{slope}$.

than did the total number of individuals within the sampling area (Figure 6 bottom). In contrast to the single-cohort samples used to estimate N_b, these spatial samples included individuals of all ages. Therefore, these estimates relate more directly to effective population size per generation (N_e) than to the effective number of parents in 1 year (N_b).

Discussion

The ratio of effective size to census size in natural populations has attracted a great deal of attention in the literature (Nunney 1993; Frankham 1995; Palstra and Fraser 2012). We have used a number of eco-evolutionary models in what we believe is the first study that formally accounts for both age structure and spatial structure in estimating this key ratio. Because all models are abstractions of reality, before considering biological insights gained we review some methodological challenges associated with this study.

Methodological Issues

What is the Sample Size?

Parentage analysis was the key to obtaining age- and sex-specific estimates of the mean (b_x) and variance (V_x) of fecundity, and their ratio (ϕ_x). It is rare to find empirical estimates of ϕ_x stratified by age and sex in natural populations; see Clutton-Brock (1988) for some early examples. For convenience rather than biological reality, it is often assumed (e.g., Felsenstein 1971; Wang et al. 2010; Waples et al. 2013) that individuals of each age and sex behave like mini Wright–Fisher ideal populations with random reproductive success, which is equivalent to assuming that $\phi_x \approx 1$. Genetic parentage methods should facilitate collection of more empirical data for ϕ_x, just as it should for other vital rates. It is difficult, however, to convert estimates based on samples into values that can be used to estimate effective size, because the variance-to-mean ratio is very sensitive to the mean. To use vital rates to calculate N_e when generations overlap, ϕ_x must be scaled to the mean fecundity (b_x) for each age and sex (Waples et al. 2011) using the appropriate sample size. This can involve a large expansion factor (see Table 2) and results can be crucial to subsequent analyses, so some time was devoted to this step in the text. Because of space limitations, important details about these and other analyses can be found in Detailed Methods in Supporting Information.

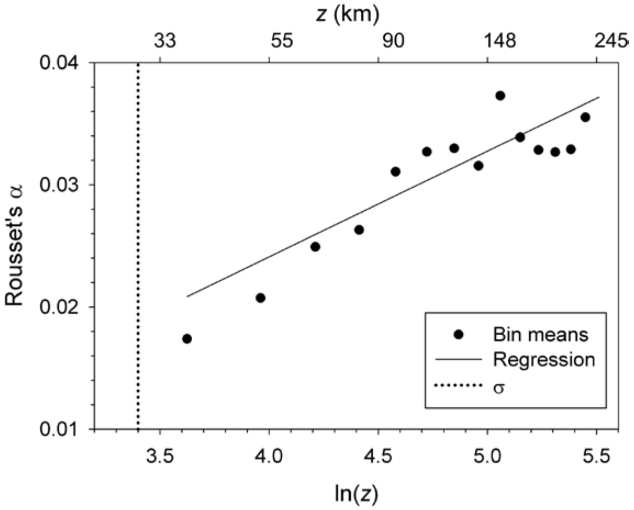

Figure 5. Relationship between Euclidean distance between pairs of individual black bears (z) and Rousset's measure of pairwise genetic differentiation (α). Data points are means for all comparisons for which z falls within bins having means of z = 37.5, 52.5…232.5 km. The fit (r^2 = 0.84) of the regression line to the data points indicates that the increase in α with ln(z) is nearly linear. As recommended by Rousset (2000), we calculated the regression after omitting comparisons for which z was less than the mean axial dispersal distance (σ = 30; vertical dotted line). These data are means for all years combined; see Supplementary Figure S11 for separate results for time periods 2002–2003, 2006–2007, and 2009–2010.

Reproductive Constraints

The most widely used demographic models for calculating effective size in species with overlapping generations (Felsenstein 1971; Hill 1972; Waples et al. 2011) all assume that 1) reproduction and survival are independent across time periods, and 2) an individual can produce an arbitrarily large number of offspring in one time period. Neither is true for Michigan black bears, nor for many other organisms. Adding the constraint that no female can produce more than 4 cubs in one litter that survive to age 1 year reduced the point

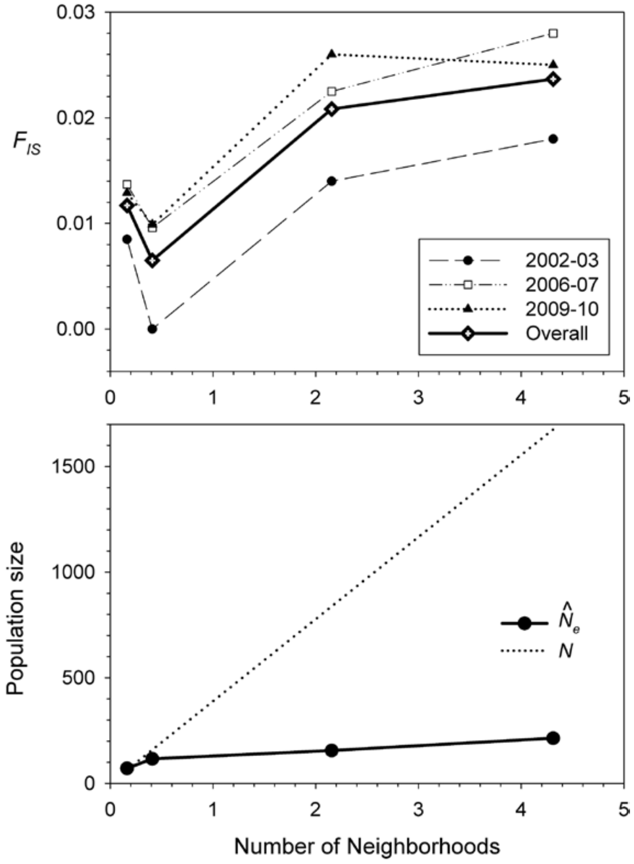

Figure 6. Effects of the geographic scale of sampling on genetic indices. The x axis shows how many genetic neighborhoods (spatial area = 8547 km²) each sample covers. Each data point summarizes data for 10 random samples drawn from areas of the indicated size, randomly placed across the study area (details in the Methods section). Top: F_{IS} is a measure of departures from Hardy–Weinberg equilibrium (positive values indicate a deficiency of heterozygotes). Results are shown separately for bears collected in 3 time periods and across all years ("overall"). Bottom: $\hat{N}_e$ is an estimate of effective size based on the LD method. Results shown are harmonic means across 3 time periods; see Supplementary Figure S12 for separate results for each time period. The dotted line shows how the total number of age 1+ individuals should increase as a function of the geographic scale of sampling, based on mean density of bears/km².

estimates of ϕ_x for females of most ages (Table 2; Supplementary Figure S9). Whether similar constraints apply to males is more speculative, but our analyses showed that this factor could potentially reduce ϕ_x for older bears.

Waples and Antao (2014) reported that a species' demography has a profound effect on the consequences of skip breeding: N_b is reduced only modestly (up to about 10%) in species with low fecundity and high adult survival but can be strongly reduced (by 50% or more) in species with high fecundity and high juvenile mortality (e.g., marine fish or sea turtles). Our results for black bears are consistent with this: N_b dropped only by an estimated 9% after accounting for skip breeding, and the demographic estimate of N_e rose by just 3%.

Stable Population Assumption

The common assumption of constant population size and stable age structure, which underpins most of our demographic analyses, is never fully met in populations in nature. Several simulation studies (Waples et al. 2011, 2014; Waples and Antao 2014) have shown that the AgeNe model is robust to random demographic stochasticity. Furthermore, Felsenstein (1971) showed that his related model accurately predicts N_e for populations that are growing or declining at a constant rate.

Estimating Effective Size

Demographic and genetic estimates of effective size both depend on variation in reproductive success among individuals, and these estimates are most meaningful when they reflect one full generation (i.e., production of offspring that survive to reproductive age). The genetic estimates of N_b were from bears of various ages sorted into cohorts. If survival is random after the earliest age at sampling (1.75 years in our study), then the estimate of N_b does not depend on the age of sampling. However, if family-correlated mortality occurs at subsequent ages, then the estimate of N_b can vary depending on when a cohort is sampled. We expect that if there is any such effect in our black bear dataset it is small, because each of the cohort samples included primarily adults of mixed ages.

Parent–Offspring Dispersal

A key parameter in Wright's neighborhood size model is the distance between the birthplace of the parent and birthplace of the offspring. We do not have data on birthplaces and have substituted instead the location of deaths, as recorded in the harvest data. The difference in the location of death of a parent and its offspring should be a good proxy for the parent–offspring dispersal distance, provided that the location of death is a reliable indicator of where that individual reproduced. This in turn should be generally true if bears rapidly establish territories or home ranges after leaving their natal area and remain there throughout their reproductive lifespan.

This is not necessarily the case with black bears, as animals can continually move across the landscape as long as they live. Young males often reproduce at locations near their birthplace and disperse farther only when older; furthermore, pairs that mated at an early age were significantly more related to one another than random pairs in the NLP population were (Moore et al. 2015). Our empirical data show considerable inter-male variation in parent–offspring dispersal distance as a function of age at death (Figure 4) but do not provide a clear basis for quantitative adjustments. To the extent that young males tend to reproduce closer to their birthplace than where they eventually are harvested, male demographic neighborhood size (and hence overall demographic neighborhood size) will be overestimated. This factor could explain why our estimate of NS_{demo} was slightly higher than our estimate of NS_{geno}. The fact that younger males have greatly reduced reproductive success compared to older males (Figure 3) should reduce potential biases associated with this effect.

Uncertainty

Some of our analyses, especially estimates of N_e/N using both demographic and genetic data, integrate a number of semi-discrete analytical steps into an overall result. This makes it challenging to quantify the degree of uncertainty associated with final estimates. Rather than attempting this step, we have provided confidence intervals for individual analyses for which they can be readily computed (e.g., Table 5, Figure 4, Supplementary Figures S7 and S8) and, in other analyses, we have provided results for multiple scenarios that bracket a realistic range (e.g., Tables 4 and 6, Supplementary Tables S4–S6).

Biological Insights

Our results illustrate practical application of a number of recently developed methods of data analysis that provide novel insights into the biology of Michigan black bears. These innovations could be applied more widely to age-structured species that also exhibit spatial structure. We elaborate these technical matters in the Supplementary Information.

Mortality

We estimate that harvest represents 80% of total annual mortality for males and 64% for females. The remainder, attributed to natural mortality, is estimated as 7.5% per year for males and 9.7% per year for females. These latter figures agree well with nonharvest mortality estimates based on radio-collared black bears in the NLP (7% for adult males and 10% for adult females; Etter and Mayhew 2008). Our data suggest that annual survival might increase for males up through about age 8–10 years and might decline thereafter, but sample sizes of older males are not sufficient to demonstrate this conclusively.

Fecundity

Fecundity rises sharply and steadily for males aged 4–10 and might decline substantially thereafter (Figure 3), but (again) sample sizes of older male bears are not sufficient to draw any firm conclusions about reproductive senescence. Consistent with data for black bears from other areas (Kolenosky 1990; Stringham 1990), as well as with results for Michigan bears based on over-winter den checks (Etter et al. 2002), estimated female fecundity also increased steadily, albeit less sharply, from ages 2 to 10 years. Sample sizes of older females are larger than those for males and do not provide any evidence for reproductive senescence (Figure 3).

We found strong evidence for overdispersion of reproductive success ($\phi_x > 1$) in both sexes; however, after stratifying the parentage assignments by age and sex, small sample sizes limited our ability to draw detailed conclusions about changes in ϕ_x with age. Still, the variance in offspring number for adult females can be several times as large as the mean, and for older males perhaps 20 times or more as large as the mean. Accounting for litter size constraints modulated our estimates of ϕ_x substantially in females and to some extent in males. This result illustrates the importance of collecting more empirical data from natural populations on this key life-history parameter.

Dispersal and Spatial Structure

We found strong and concordant evidence for isolation by distance in both demographic and genetic datasets. Genetic studies of black bears in a much larger area (>10^6 km^2) in Ontario, Canada also detected isolation by distance and weak clinal variation (Pelletier et al. 2012). Therefore, the phenomenon may be common in the species.

Consistent with patterns reported by Moore et al. (2014, 2015), males dispersed much farther than females at most ages (Figure 4). As a consequence, the demographic estimate of *NS* was almost 3 times as large for males (Table 6). Although sex-biased dispersal is well documented for many species (Clobert et al. 2009), it is not commonly dealt with in an isolation-by-distance framework. As best we can determine, this is the first study to calculate sex-specific neighborhood sizes and to use Wright's (1938) sex-ratio adjustment to calculate an overall neighborhood size that formally accounts for different dispersal rates of males and females. Our demographic estimate NS_{demo} = 100 (range = 89–112 depending on estimated density) is close to the genetic estimate NS_{geno} = 85 (range 68–109 depending on the time period) based on the slope of the α versus ln(*z*) relationship. Such close agreement between genetic and demographic estimates of *NS* is unusual (Rousset 2000).

This analytical framework is expected to perform best when mean axial dispersal distance (σ) is "small" and most sampling occurs within an area of 10σ×10σ (Rousset 2000). For Michigan black bears, our estimate of σ is 30 km so 10σ×10σ = 90 000 km^2—over twice as large as the study area. Thus, our experimental design fits comfortably within the recommended parameter space.

Genetic and Demographic Estimates of Effective Size

Adult census size (N_A) is the most widely used metric for computing the N_e/N ratio (Nunney and Elam 1994; Frankham 1995), and our "best" demographic estimate of $\hat{N}_{e(demo)}/N_A$ is 0.52 for Michigan black bears (Scenario V in Table 4). This is consistent with predictions in the literature. Nunney (1993) proposed that N_e/N_A should generally be larger than 0.5 but asymptotically approach 0.5 for long-lived species.

Often it is not possible to reliably distinguish adults and juveniles in the field, so some authors use total census size in the denominator of the N_e/N ratio. That also is the ratio we used to compute effective density, because the mark-recapture estimates of abundance applied to bears age 1 and older. Our best demographic estimate of N_e/N was 0.28. Harris and Allendorf (1989) modeled effective population size in the grizzly bear (*Ursus arctos*) and evaluated the performance of a variety of demographic estimators. The formula by Hill (1972), which forms the basis for the AgeNe program, performed well in their study, and $\hat{N}_{e(demo)}/N$ ranged from 0.26 to 0.36 for a variety of demographic and management scenarios, which nicely brackets our estimate for Michigan black bears. Harris and Allendorf (1989) also found that high male variance in reproductive success was a major factor reducing N_e compared to *N*. Using the estimator by parentage assignments (EPA) method developed by Wang et al. (2010), Kamath et al. (2015) reported substantially higher estimates for Yellowstone grizzly bears ($\hat{N}_{e(geno)}/N$ = 0.42–0.66), which they tentatively attributed to the small size of the population. However, the EPA method assumes that $\phi_x = 1$ for each age and sex, which means that the method will overestimate N_e when variance in reproductive success is overdispersed within ages. For our black bear data, $\hat{N}_{e(demo)}$ declined by 18% (from 562 to 461) and by 24% (from 562 to 427) when the generic assumption that $\phi_x = 1$ was replaced with the "middle" or "high" empirical estimates (compare Scenarios I, II, and III in Table 4).

Our best demographic estimate of N_e (475 from Scenario V, which uses "middle" estimates of ϕ_x and accounts for reproductive constraints) is close to the 500 value that is often cited as a rule-of-thumb for an effective size required to maintain genetic diversity over the long term (Franklin 1980; Soule 1980). This is encouraging but does not provide much of a buffer, considering various sources of uncertainty in the analyses. Furthermore, the not-quite-significant declining trend in genetic estimates of N_b for cohorts born between 1998 and 2009 indicates that it would be prudent to continue genetic monitoring of this population.

Genetic estimates of generational N_e in species with overlapping generations are confounded by age structure, and these complications can be minimized by focusing instead on estimating the effective number of breeders per year (N_b). N_b is much more sensitive than N_e to overdispersed variance in reproductive success. Our best demographic estimate (from Scenario V) of the N_b/N_e ratio is 0.44. We can compare that with predictions based on the relationship between demographic N_b and N_e in a large number of species with diverse life histories. In the simplest predictive model, Waples et al. (2013) found that the following relationship explained two-thirds of the variation in N_b/N_e across 63 species: N_b/N_e = 0.485 + 0.758*log (*AL*/*AM*), where *AL* at sexual maturity. Using averages across male and female black bears, *AM* = 2.7 and *AL* = 18.3, leading to a predicted N_b/N_e ratio of 1.11. This model assumed that all $\phi_x = 1$, and the predicted value is reasonably close to the N_b/N_e ratio calculated under Scenario I, where N_b is expected to be very slightly larger than N_e in black bears (Table 4). However, this simple model dramatically overestimates the best estimate of N_b/N_e (0.44) in black bears, which explicitly accounts for empirical evidence of overdispersion in reproductive success.

Because each bear was aged, we were able to reconstruct 12 consecutive cohorts of genetic samples (Table 5). After accounting for age structure, adjusted $\hat{N}_{b(geno)}$ was on average 16% less than demographically estimated N_b. In theory (Waples et al. 2014), a single-cohort sample analyzed with the LD method should provide an estimate of the harmonic mean of N_b and N_e, but the raw $\hat{N}_{b(geno)}$ estimates averaged 32% lower than this. Two factors are likely responsible for this discrepancy. First, the demographic estimates might be underestimating ϕ and hence overestimating N_b. If we use the high empirical estimates of ϕ (Scenario VI), the genetic and demographic estimates of N_b are in close agreement (geometric mean ratio = 1.05; Supplementary Table S6). However, under Scenario VI the raw genetic estimates are still on average 18% lower than the harmonic mean N_b,N_e using demographic data.

We believe that population structure explains this remaining discrepancy. Isolation by distance (as documented in the spatial analyses) creates linkage disequilibrium due to mixture when individuals from different genetic neighborhoods are combined in a sample, as was the case here when genetically estimating effective size. This "mixture LD" is interpreted as arising from drift, which downwardly biases the genetic estimates of N_b. Effects of spatial structure on genetic estimates of N_e are seen in Figure 6: as the geographic area of sampling increases, $\hat{N}_{e(geno)}$ also increases, but much more slowly, and across the entire study area $\hat{N}_{e(geno)}$ is ~220, only 45% of the demographic estimate for Scenario V (Table 4). As the geographic scale of sampling increased, F_{IS} became more positive, indicating an increasingly strong heterozygote deficit caused by the Wahlund effect. Neel et al. (2013) found a similar result in modeling 2D isolation by distance for a discrete-generation model. Collectively, these results strongly argue for caution in using raw estimates of effective size from the LD method to study populations that are continuously distributed over large areas. Geographically restricted samples might accurately reflect the genetic neighborhood size, but samples that include multiple genetic neighborhoods do not accurately reflect global N_e.

Differences in vital rates between males and females influenced annual effective size more than generational effective size. The demographic estimate of N_b was 3 times as high for females as it was for males (Table 3), reflecting 1) higher survival rates for females (hence an adult sex ratio skewed towards females); 2) stronger changes in fecundity with age in males; and 3) greater overdispersion in male reproductive success. The overall annual demographic estimate of N_b (211 for Scenario V) represents 13% of the total population size of age 1+ bears and 22% of the estimated adult population size. As noted above, comparable estimates for generational effective: census size ratios for Scenario V were over twice as high: $\hat{N}_{e(demo)} / N = 0.28$ and $\hat{N}_{e(demo)} / N_A = 0.49$. These strong effects of vital rates can be contrasted with the relatively modest effects noted above associated with skip breeding (which reduces N_b by <10% and increases N_e by <5% in Michigan black bears).

Summary

We believe that this is the first comprehensive analysis that explicitly accounts for both age structure and spatial structure in the study of ecological and evolutionary processes for a natural population. Novel features include the following:

- We show that accounting for all potential parents to properly scale sample estimates of mean and variance in fecundity by age and sex—something that is usually straightforward in controlled experiments—can be challenging in studies of natural populations and can substantially affect estimates of age-specific vital rates.
- In both males and females, we find evidence for overdispersed age-specific variance in reproductive success—a key parameter that is seldom estimated empirically but which strongly influences the ratio of annual to generational effective size (N_b/N_e). The N_b/N_e ratio, in turn, is used in adjusting genetic estimates of effective size to account for effects of age structure.
- Skip breeding and constraints on litter size are common life history traits whose effects on eco-evolutionary analyses are seldom evaluated. We show that small litter sizes in black bears constrain variance in reproductive success and keep annual N_b higher than it would be otherwise. Skip breeding has a more modest effect in decreasing N_b and slightly increasing N_e.
- Genetic estimates of N_b were consistently about 15% lower than demographic estimates, a result we attribute to genetic isolation by distance that downwardly biases the genetic estimates, which assume a single, randomly mating population.
- Because dispersal is heavily sex-biased, we calculate separate demographic estimates of neighborhood size for males and females and show that the male *NS* is about 3 times as large as female *NS*. We are not aware of other studies that have estimated sex-specific neighborhood sizes. The overall demographic *NS* (100) is comparable to the overall genetic estimate (85), which indicates that the study area includes about 4–5 neighborhoods (*sensu* Wright) of black bears.

Supplementary Material

Supplementary data are available at *Journal of Heredity* online.

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Data Availability

Data used in this study, including bear microsatellite genotypes and harvest locations and data, are available for download on Dryad (https://datadryad.org//resource/doi:10.5061/dryad.c61q0).

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