POPULATION GENETIC STRUCTURE IN THE EASTERN POPULATION OF GREATER SANDHILL CRANES (*GRUS CANADENSIS TABIDA*)

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INTRODUCTION

The Sandhill Crane (SACR; *Grus canadensis*) is the most populous of the 15 extant crane species. Their breeding range covers the majority of the continental U.S. and Canada and extends into Cuba and Eastern Siberia. Wintering areas are much more condensed and range typically from the southern and southeastern U.S. into Mexico (Meine and Archibald 1996). While not the most abundant population, the Eastern Population (EP) of greater SACRs (*G. c. tabida*) is likely the currently fastest growing (3.9% annual average growth rate; Amundsen and Johnson 2010). It is estimated to comprise approximately 60,000 birds (Kruse et al. 2012).

Historically, unrestricted harvest and substantial habitat conversion forced the EP through an extreme demographic bottleneck in the early 1900's and reduced the population to fewer than 300 birds with less than 50 known breeding pairs scattered among Stearns/Sherburne/Wright counties in central MN; Burnett, Adams/Juneau, and Marquette counties in WI; and Schoolcraft, Jackson, and Allegan/Barry counties in MI (Henika 1936, Walkinshaw 1949). Since the U.S. Fish and Wildlife Service began fall counts in the 1970's, the EP has expanded considerably as a result of conservation and environmental protection. Moreover, acclimation of birds living near humans and use of agricultural crops (primarily corn) as a food source has helped increase their population density. Not only have SACRs increased in the core refugia, but also re-colonized much of its historic range in IL, IA, OH, IN, and Ontario (ON), Canada (Meine and Archibald 1996).

Outside of the Great Lakes region, SACRs have also been spreading into the northeastern U.S. and New England states. Breeding activities have been documented in PA (1994), ME (2000), NY (2003), MA (2007), and VT (2008; Melvin 2002, 2010). Based on the extant distribution of SACRs, it is possible that these areas historically maintained viable albeit small populations, but cranes in these areas were almost certainly extirpated during the time of early European settlement in the 1600's and 1700's (Melvin 2002). It is now hypothesized that this new population was established by an eastward expansion of SACRs from the core region of the EP (Meine and Archibald 1996, Melvin 2002, 2010) but the exact source(s) remain(s) unknown.

SACRs breeding in the northeastern U.S. and New England are of special interest because this population is extremely small (probably less than 15 breeding pair spread throughout five U.S. states), but is beginning to increase and expand its range (Melvin 2002, 2008). Unlike the EP, observations of birds in the northeast U.S. and New England are widely scattered and could prevent or delay birds from finding mates to form breeding pairs (Allee effect). Comparing documented population growth patterns for the EP may be useful to predict growth patterns for SACRs breeding in the northeastern U.S. and New England because this new population is likely mirroring events that occurred in the EP as it recovered from its own population bottleneck nearly 70 years earlier.

The goal of this project was to establish genetic relationships between SACRs in core breeding areas in the EP. Specifically, we set out to determine the amount of gene flow between known refugia and attempted to determine possible source populations for areas that were re-colonized during the re-growth and expansion of the EP. We also investigated relationships between birds breeding in the northeast U.S. and New England to the EP to evaluate whether this was the sole founder of this growing population or if there could also be any contributing influence from other populations.

METHODS

Flightless SACR chicks were captured by foot pursuit until they hid and could be handled (Hoffman 1985). Colored leg bands were attached to easily identify the location of where the birds were originally banded (see www.bandedcranes.org). A U.S. Geological Survey band was placed above the toes or within the colored leg bands to allow permanent identification. Additionally, a small blood sample was collected from jugular or tarsal veins for genetic analysis.

Sample locations were distributed throughout the known range of the EP (Fig. 1, Table 1). We focused on sampling areas that are likely remnant populations that served as refugia for this population during the bottleneck (sites 2, 3, 4, 5, and 7), including Briggsville, WI where the International Crane Foundation has been banding and monitoring SACRs since 1991. Additional areas that were likely re-colonized following the bottleneck included sites 1, 6, 8, 9, 10, and 11. Combined, these samples form a well-distributed representation of SACRs in the EP. The samples in table 1 were compared to other samples collected for various other projects:

- one SACR that was found as an injured hatch year chick in ME in 2007 and now resides at the Brandywine Zoo in Wilmington, DE
- twenty-five samples from flightless SACR chicks in IL northeast of Chicago collected by Jeff Fox (Illinois Natural History Survey and University of Illinois at Urbana-Champaign)
- eleven samples from injured or abandoned flightless chicks in southeast WI raised by local rehabilitator Pat Fisher (Appleton, WI)
- five samples collected from adult SACRs in OH by Dave Sherman (OH DNR)

Combined, a total of 256 SACR samples were included in this study.

Amplified Fragment Length Polymorphisms (AFLP; Vos et al. 1995) fingerprints of each individual were used to estimate various population genetic parameters. The AFLP procedure samples neutral, anonymous loci throughout the genome and is capable of distinguishing between nearly identical strains of bacteria and plants. Empirical studies and our own experience indicate that scoring and reproducibility of the AFLP technique approaches 100 percent. We generated 210 loci with one AFLP primer pair, of which 158 showed acceptable baseline resolution and were capable of being consistently scored as present or absent. Of these, 141 of 158 loci (89%) were polymorphic and were used in all analyses.

We inferred population genetic structure with a Bayesian Monte Carlo Markov Chain (MCMC) method implemented in Geneland 4.0.3 (Guillot et al. 2005, Guillot and Santos 2010). This method uses a Poisson-Voronoi tessellation procedure that incorporates genetic and coordinate (spatial) data to assign genetically-related individuals into clusters. The use of spatially explicit genetic information allowed us not to assume that each sampling locality represented a distinct population. Instead, this procedure allowed us to exploit both genetic and geographical information to estimate the most likely number of genetically homogenous clusters. Additionally, this technique provides a means to identify dispersing individuals. Based on this strategy, we performed 1,000 independent runs in Geneland each with 1,000,000 iterations and a burn-in of 10,000 iterations. The variation of cluster membership between

individuals in all MCMC replicates was visualized by constructing a topological network using unweighted average distances between clusters (UPGMA). The resulting topology does not show phylogenetic history, but rather demonstrates overall relationships between individuals based on shared membership to the same genetic cluster.

We tested for isolation-by-distance (IBD) by first calculating pairwise F_{ST}, a relative measure of genetic similarity (Wright 1951), between each pair of sample sites in AFLP-SURV 1.0 (Vekemans 2002). Samples from Southeast WI, OH, NY, PA, and ME were excluded from this analysis because it was difficult to find a meaningful geographic centroid between sampling locations which were highly scattered. Moreover, the small sample sizes of these locations would not allow us to reliably estimate F_{ST} between these and other sampling locations. These pairwise comparisons of F_{ST}, were plotted as $F_{ST}/(1-F_{ST})$ against the natural logarithm of the distance (Km) between each sampling location (Roussett 1997). A Mantel Z-test with 100,000 replicates was used to estimate any correlative significance between $F_{ST}/(1-F_{ST})$ and the log of geographic distance. The slope of the IBD relationship was estimated with reduced major axis regression and tested for statistical significance with 100,000 bootstrap re-samplings. The same sample groupings were also used to calculate inbreeding coefficients (F_{IS}).

Private loci (calculated in Genetic Data Analysis 1.1; Lewis and Zaykin 2002) show another level of differentiation that differs from F_{ST} because it reflects loci where every bird in the population shows the presence or absence of a specific locus and all other birds from other populations show the opposite state. Populations containing more private loci exchange fewer migrants than those without private loci (Slatkin 1985). In contrast to F_{ST}, private loci may also be less sensitive to sample size and are therefore more suitable for those sites not included in the EP analysis.

RESULTS

Re-sightings of color-banded SACRs occurred throughout the migratory flyway suggesting extensive mixing of breeding populations on migratory stopover and wintering areas (Fig. 1). For ICF's long-term study area near Briggsville, WI, seasonal banded-bird re-sightings suggest strong natal philopatry for chicks hatched in this area. Males were highly philopatric and 77% (n = 22) nested for the first time within four km of their natal area. Females were more dispersive, and 69% (n = 16) nested for the first time within eight km of their natal site. The furthest documented dispersal thus far recorded was a female that nested 57 km away from her natal area. Even non-breeding birds exhibit strong philopatric tendency in successive years. The maximum post-fledgling distance of a previously banded flightless chick was a one-year old bird found dead 200 km north of Briggsville. In contrast, most radio-tagged individuals stayed within 50 km of their natal area. For those cranes banded outside of Briggsville, few re-sightings have occurred. However, one female banded in Sherburne NWR (MN) in 2007 later paired and nested 20 km SE of her natal area.

Amplified Fragment Length Polymorphisms provided a precise genetic method to measure patterns of recovery and re-colonization in the nascent EP. Based on pairwise F_{ST} between sample sites and overall F_{ST} for the population as a whole in the EP, we documented statistically significant levels of genetic structure (Table 3). However, there was no evidence for isolation-by-distance in the EP (Z=11.624, one-sided p=0.25; Fig. 2). Although the calculated slope of the IBD relationship was positive, it was also not significant (slope=0.25, R^2=0.012, 95% CI = -0.3746, 0.3613). Expected heterozygosity was highest among chicks sampled from the northeastern U.S. (Table 2). The northeastern U.S. also had the highest number of private loci overall and when scaled for sample size (Table 2).

Analysis of Geneland replicates partitioned EP SACRs into a modal K=5 clusters (56% of 983 iterations supported five genetic clusters; 17 replicates failed to converge based on a Gelman and Rubin MCMC convergence diagnostic). Overall, the topology of relationships showed consistency with individuals sampled at the same location (Fig. 3). One branch contained both sample sites from the Lower Peninsula

of MI and another contained the four chicks banded in northwestern PA in 2011. A third branch contained the birds banded in Sherburne MN, Crex Meadows WI, Seney MI, southeast ON, and the injured chick from ME. A fourth branch contained only the SACRs sampled in Necedah WI. The last branch contained all Briggsville WI cranes along with birds banded in IL, OH, Southeast WI, Eastern PA, and NY. The birds sampled in NY grouped with the birds from southeast WI. One OH bird and the chick from eastern PA grouped with the birds from northeastern IL, suggesting that OH adult may be a migrant from this location.

CONCLUSIONS

This is the most complete genetic sampling and analysis of SACRs undertaken throughout the known range of the EP. Sampling at locations that survived the demographic bottleneck (Henika, 1936; Walkinshaw, 1949) as well as nearby areas that were re-colonized (pers. comm. from refuge staff and landowners) allowed us to investigate historic and more contemporary relationships within and between these areas. Most SACRs grouped with others sampled in the same locality. This suggests a high degree of natal philopatry that is also supported by statistically significant levels of genetic differentiation and repeated re-sightings of banded and radio-tagged birds near their natal areas (ICF, unpublished data). These results, despite extensive mingling of birds from different breeding areas on wintering grounds (Fig. 1), suggest that mate choice occurs between individuals from similar geographic areas. If mating were independent of breeding geographic locality (i.e. random mate choice), more extensive genetic population homogenization should be observed but this was not the case. While most birds in this study had not yet reached adulthood at the time of sampling, given the observed magnitude of natal philopatry, their parents most likely originated from the same area.

Other relationships between genetic composition and sample site are more complex. The connection between sampling locations in the Lower Peninsula of MI is not surprising given their proximity to each other. The small number of birds that remained there during the demographic bottleneck in the early 1900s and the high probability of genetic exchange both historically and contemporarily maintained a high level of genetic relatedness. However, evidence of natal philopatry was still detectable in our analysis as most individuals clustered into geographically distinct areas. The single branch containing only SACRs from Necedah WI suggests isolation from all other sampled EP groups. This result is surprising considering its location in the center of the range of the EP. Banded individuals from Necedah have been observed in Briggsville WI and birds banded in Briggsville have also been observed in Necedah. There has never been, however, observation of these birds establishing a territory in the opposite area. While both areas have wetland complexes, the upland areas surrounding these wetlands are very different. Necedah contains native grasslands that are heavily managed, while Briggsville uplands are dominated by row crop agriculture. These differences may affect how birds hatched in different areas view these alternatives in terms of habitat quality, but the extent of habitat imprinting remains unknown in SACRs.

The branch with birds sampled at Sherburne MN, Crex Meadows WI, Seney MI, southeast ON, and the bird from ME suggest the presence of an east-west genetic relatedness across the northern known range of the EP. A similar trend across the southern range of the EP was also observed with SACRs sampled from Briggsville WI, OH, IL, southeast WI, and birds from Eastern PA and NY. This suggests both historic and extant east-west movements along latitudinal lines an interpretation also supported by Jones et al. (2005) who found close relationships between Rocky Mountain and EP greater SACRs using microsatellite data.

Our detection of these relationships is likely more historic than contemporary as SACRs have long generation times and we are using nuclear markers which have slower rates of recombination. This suggests that individuals surviving the bottleneck could serve as partial genetic reservoirs and pass historic assemblages of alleles on to their offspring. A similar phenomenon has been observed in a

bottlenecked population of white-tailed eagles (Hailer, et al., 2006). Currently, most pairing of SACRs appears to occur between birds from similar breeding areas. During a demographic bottleneck, flexibility in mate choice during times of low population densities (Allee effect) could lead birds to form pair bonds anytime and place during their annual cycle. Pairing of birds from different breeding areas during the bottleneck may help explain why certain SACRs sampled from northeastern areas clustered with those individuals from the Great Lakes areas. This could also be indicative of long-range dispersal and founding events. However, more samples from the northeastern areas will be needed to properly address these hypotheses.

Overall, the results of this study suggest there is substantial population genetic structure in SACRs from the EP and northeastern U.S. Topological relationships of clustering individuals show significant genetic differentiation between breeding areas throughout the EP and less differentiation among birds at specific locations. This result is consistent with the observed philopatry of SACRs. The next step would be to test this genetic model to predict a probable location of origin for a bird sampled from the EP on migratory staging or wintering areas. We have successfully performed this predictive analysis in SACRs sampled from the Pacific coast population (Hayes, Berres, et al., in prep). For Pacific coast populations of SACRs, however, reduced population sizes allowed us to use a relatively small number of individuals (a minimum of 10) from each breeding population to serve as representative standards to assign birds that were sampled on wintering areas. To accomplish this same goal with the EP, more representatives from breeding areas throughout the EP will be necessary. Additionally, increased sampling from northeastern U.S. and New England breeding areas would further augment and the limited sampling conducted previously in these areas. Increased sampling and banding (including telemetry) in this new Atlantic Flyway would aid in continuing to learn more about its migratory habits and genetic relatedness to EP and other populations.

Some SACRs that hatched in the northeastern U.S. associated with multiple branches that also contained known EP individuals. One interpretation suggests that émigrés from the EP helped establish this new population. However, birds sampled from Northwestern PA were always clustered together suggesting the possibility of additional – and as yet unknown – source population. The largest magnitude of expected heterozygosity and number of private alleles were both present in birds sampled from the northeastern U.S. These results are surprising given that this population is almost assuredly newly founded and although currently small, apparently continues to expand. Typically, founder effects from a primary source population lead to reduced heterozygosity compared to that source or other populations (Alacs et al. 2011, Tang et al. 2003). Additional samples would further define these relationships to other parts of the EP and perhaps identify as yet unknown genetically distinct populations.

One possible explanation for increased levels of heterozygosity and private alleles in northeastern SACRs posits that these birds come from a remnant population in the northeastern U.S., New England, or possibly Canada. Melvin (2002, 2008) concluded this to be an unlikely scenario as northeastern (including New England) populations were extirpated in the late 1600's. Another possibility is that multiple population sources contributed individual SACRs that re-colonized this area and this led to continued outcrossing between these populations. This interpretation is consistent with the estimated clustering relationships, each of which also exhibited an excess of heterozygotes (i.e. $F_{IS} < 0$). While the EP was expanding and re-colonizing in the Great Lakes in the 1970's, birds in the eastern portion of the Mid-Continent Population (MCP) were also spreading east around James Bay from Ontario into Quebec (Ouellet and Bourget 1975). Birds in eastern Ontario measure morphologically as *G. c. rowani* (Canadian subspecies) (Krapu et al. 2011) and tarsus measurement of a dead bird from Quebec were also indicative of *G. c. rowani* (Ouellet and Bourget 1975). The expansion of SACRs south and east into southern Quebec, New Brunswick, and Newfoundland/Labrador has been occurring since the 2000's. Data from e-Bird and collected sightings from the American Birding Association (Bannon et al. 2000, 2001, 2002, 2003, 2005; McTavish 1999, 2002, 2003, 2006, 2007, 2008; Dalzell 2009, 2010) document

an increasing number of pairs, nests, and flocks throughout eastern Canada (Fig. 4). This recent population expansion may have continued south toward New England.

Determination of the origin of these populations is important for the management concerns of the Mississippi Flyway Council. For example, if a *G. c. rowani* is harvested east of the Mississippi River, it could be erroneously suggested that this bird is from the MCP. Recording current movements of *G. c. rowani* through the northeastern U.S. including New England and from Hudson's and James' Bay is critical to a well-developed management plan for the EP and growing Atlantic Flyways of SACRs. How many subspecies are using this new Atlantic Flyway for SACRs remains unknown. General morphological appearance suggests that birds breeding in NY and PA are greater SACRs (*G. c. tabida*; Hayes, personal observation). However, no adult cranes in the Atlantic Flyway have been measured. As both Canadian and greater subspecies migrate through NY in the fall (Fig. 5 [a, b]), it is possible that SACRs from both the EP and MCP assisted in the re-colonization of the northeastern U.S. and New England.

RECOMMENDATIONS

Based on the results of this study, we offer several recommendations for further investigation into SACRs in the Northeastern U.S. and New England:

1) Continue and increase genetic sampling efforts of SACRs breeding at sites distributed throughout the Northeastern U.S. and New England. An increased number of samples will strengthen our analytical model and enable better determination of genetic relationships between the growing Atlantic Population, EP, and MCP.
2) Initiate genetic sampling of SACRs breeding in Eastern Canada (Ontario and Quebec) and Maritime Provinces (New Brunswick and Labrador). Acquisition of these samples would assist in determining the genetic relationships between this expanding population with the EP and Atlantic Populations.
3) Initiate a widespread banding and satellite telemetry study on birds breeding or hatched in PA, NY, ME, VT, and MA to determine migratory routes and wintering areas for this population. Trapping of birds and deployment of transmitters at migratory stopover areas in central NY and northwestern PA would allow estimation of the proportion of migrants to resident breeders that use these areas during fall migration. Trapping of birds and deployment of transmitters at wintering areas in the northeastern U.S. (e.g. NJ) would help determine breeding locations for wintering populations that use the northeastern U.S. during part of their annual cycle.
4) Hayes et al. (in prep) showed that SACRs banded and genetically sampled on wintering areas in California could be assigned to specific breeding populations in CA, OR, British Columbia, and AK using these methodologies described herein. We advocate the continued sampling of SACRs on wintering areas throughout the EP and northeastern U.S. to additionally verify and validate the results generated by the genetic analyses.

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Table 1. Sample locations and numbers for greater SACRs in the Eastern Population banded by the International Crane Foundation.

Location Number	Location Name	Sample Dates	Number of chicks sampled	Number of families sampled
1	Sherburne National Wildlife Refuge, central MN	7/9 - 7/12/2007	11	10
2	Crex Meadows, Fish Lake, Amsterdam Slough State Wildlife Areas, northwestern WI	7/13 - 7/16/2007	8	8
3	Briggsville, central WI	1996 – 2011*	121	60
4	Necedah National Wildlife Refuge, central WI	2000**	23	16
5	Waterloo State Recreation Area, southeast MI	6/16 - 6/18/2008	14	10
6	Gun Lake Tribal Lands, southwest MI	6/20 - 6/23/2008	10	7
7	Seney National Wildlife Refuge, Upper Peninsula MI	7/5 – 7/8/2009, 7/12 – 7/15/2010	7	6
8	Thessalon and surrounding areas, southeastern ON	7/5 – 7/8/2009	13	10
9	Central NY (Montezuma NWR and surrounding areas)	6/16 – 6/20/2011	2	2
10	Northwestern PA (Pymatuning Lake and surrounding areas)	6/21 – 6/26/2011	4	2
11	Northeastern PA (Dushore)	6/27/2011	1	1

Table 2. Genetic diversity estimates from AFLPs by sampling location for SACRs in the EP.

Location	N[a]	n[b]	PL[c]	%PL	PrL[d]	He[e]	S.E.	Avg frag/ind.[f]	PL/N	PrL/N
Sherburne NWR, MN	11	158	77	48.7%	1	0.142	0.014	50	7.00	0.09
Crex Meadows SWA, WI	8	158	74	46.8%	0	0.155	0.014	49	9.25	0.00
Briggsville, WI	121	158	61	38.6%	6	0.134	0.013	40	0.50	0.05
Necedah NWR, WI	23	158	69	43.7%	1	0.127	0.012	46	3.00	0.04
Northeast IL	25	158	72	45.6%	5	0.158	0.014	45	2.88	0.20
Waterloo SWA, MI	14	158	74	46.8%	0	0.147	0.013	52	5.29	0.00
Gun Lake, MI	10	158	72	45.6%	1	0.133	0.013	47	7.20	0.10
Seney NWR, MI	7	158	73	46.2%	1	0.144	0.013	49	10.43	0.14
Southeast ON	13	158	66	41.8%	1	0.147	0.014	47	5.08	0.08
Central OH	5	158	58	36.7%	0	0.117	0.012	42	11.60	0.00
Northeast U.S. (NY, PA, ME)	8	158	82	51.9%	7	0.197	0.016	48	10.25	0.88

Table 2 (cont.)
[a]number of individuals analyzed
[b]number of loci analyzed
[c]polymorphic loci
[d]private loci
[e]expected heterozygosity
[f]average number of fragments (loci) present in each individual

Table 3. F_{ST} estimates (above diagonal) and distances (Km, below diagonal) between sampling sites. F_{ST} estimates in bold indicate a value significantly different from 0.

	W MI	GL MI	NE IL	B WI	N WI	CM WI	SNWR MN	SE ON	SNWR MI
Waterloo, MI	0	**0.059**	**0.114**	**0.185**	**0.155**	0.024	**0.096**	**0.095**	**0.101**
Gun Lake, MI	119.240	0	**0.097**	**0.159**	**0.189**	**0.055**	**0.169**	**0.123**	**0.182**
Northeastern IL	318.053	201.850	0	**0.046**	**0.123**	**0.074**	**0.137**	**0.102**	**0.133**
Briggsville, WI	463.940	342.700	178.950	0	**0.118**	**0.134**	**0.214**	**0.136**	**0.178**
Necedah NWR, WI	520.559	403.030	244.590	62.210	0	**0.075**	**0.143**	**0.089**	**0.068**
Crex Meadows, WI	782.460	664.280	522.810	341.289	278.780	0	**0.058**	0.035	0.042
Sherburne NWR, MN	842.950	733.123	565.580	387.610	324.820	91.670	0	**0.088**	**0.073**
Southeastern ON	439.850	443.530	563.410	566.140	584.850	711.470	800.140	0	0.014
Seney NWR, MI	461.160	405.890	451.630	400.190	406.020	509.420	599.980	200.630	0

Fig. 1. Banding locations and re-sightings of banded SACRs during migration and on wintering areas.

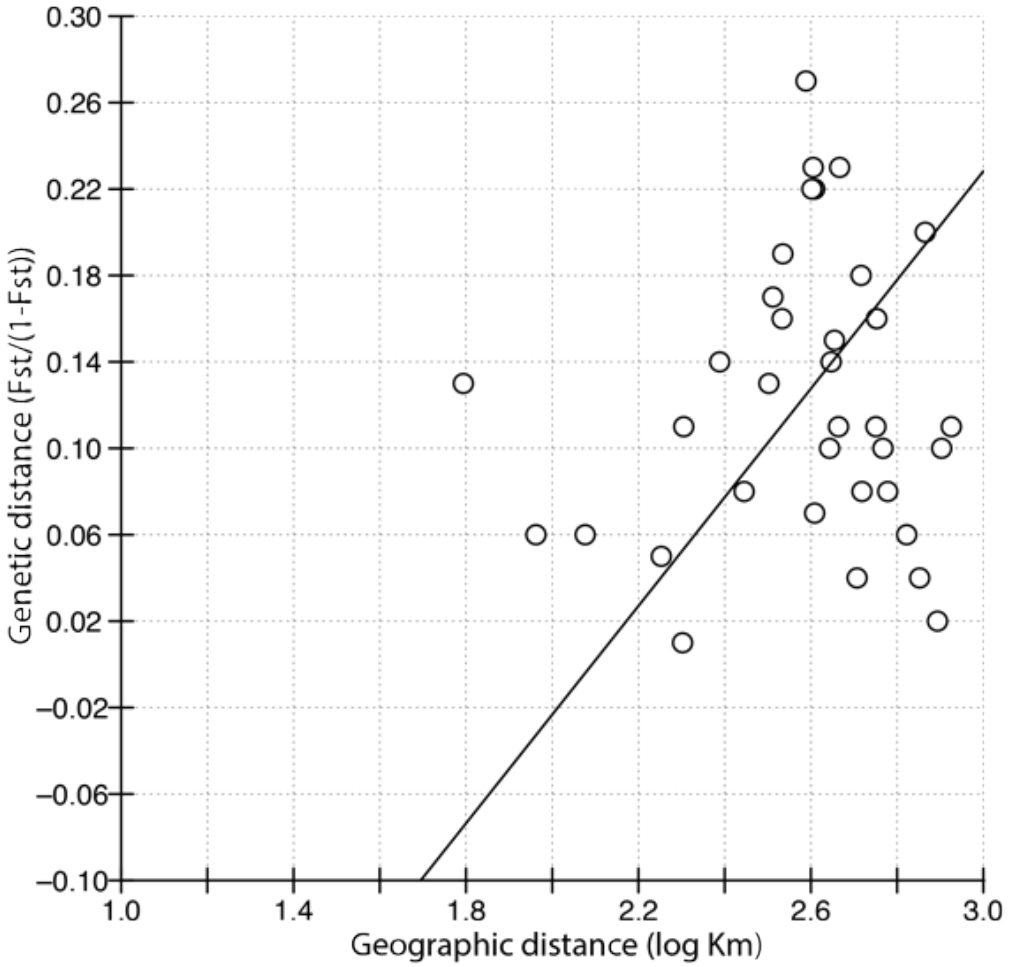
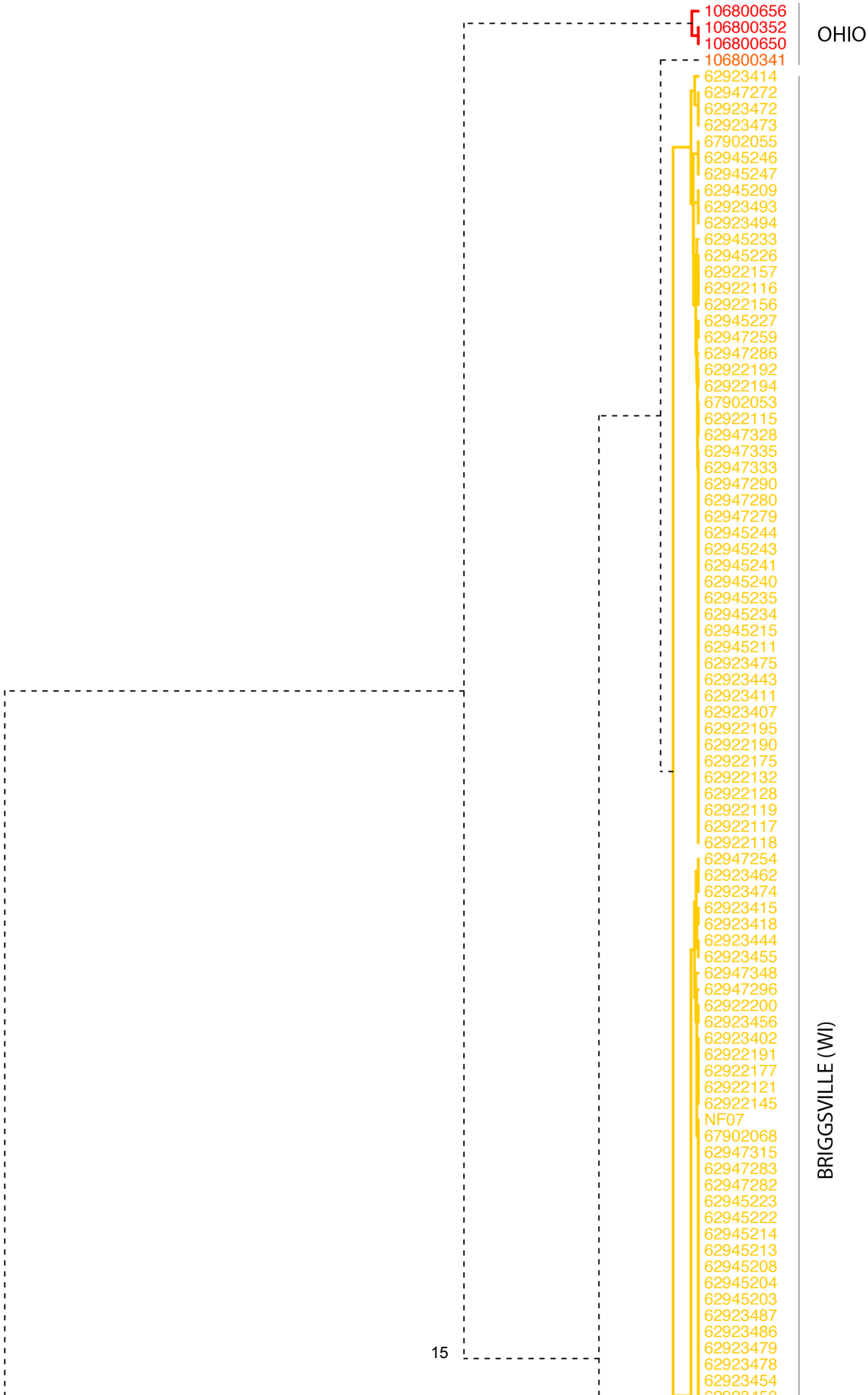


Fig. 2. Relationship between pairwise F_{ST} and log of geographical distance (Km) for sampling sites in the EP (excluding southeast WI, northeast U.S., and OH).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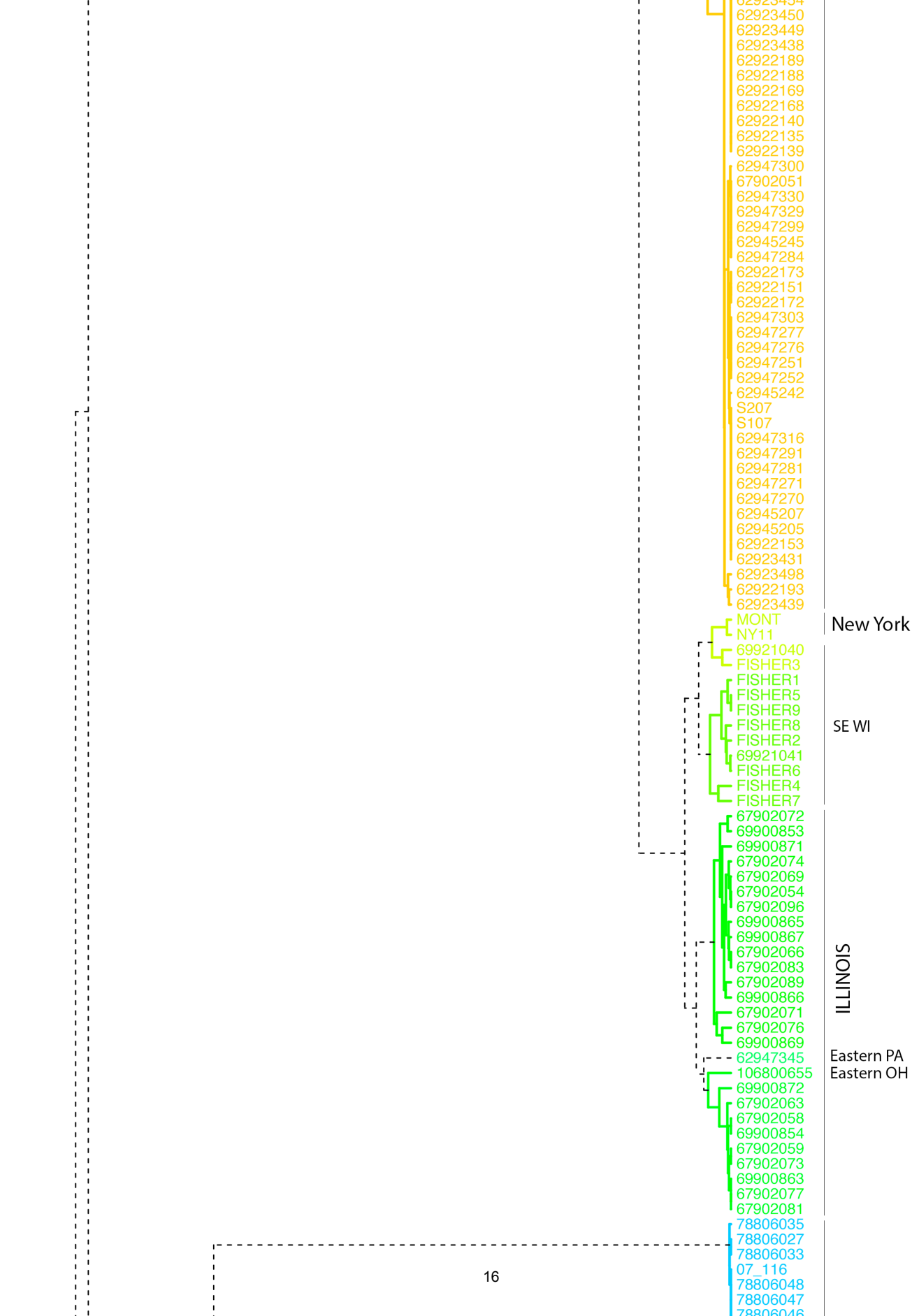
BRIGGSVILLE (WI)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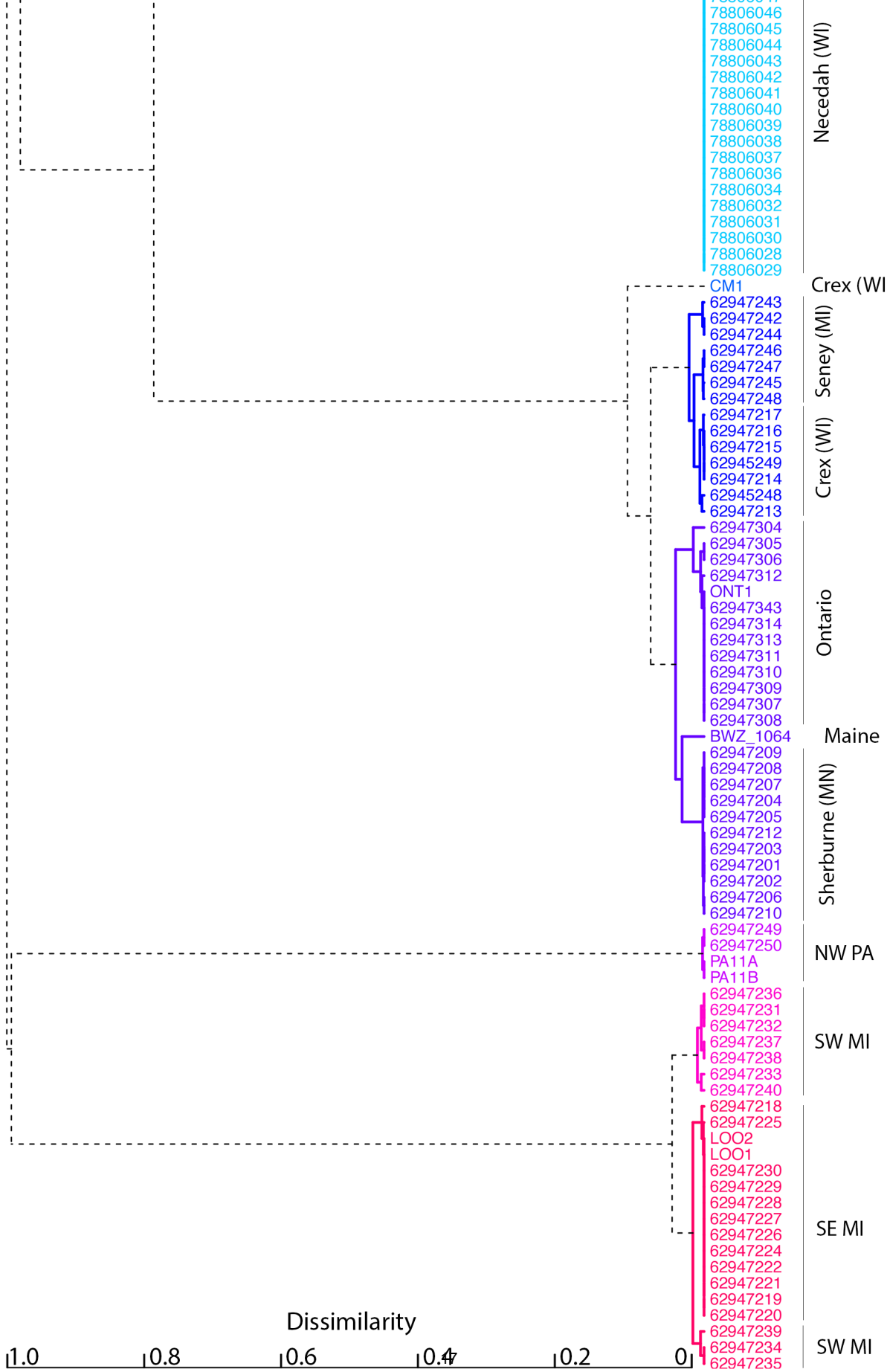
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PA11B
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SW MI
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LOO2
LOO1
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SE MI
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SW MI
Dissimilarity
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0.6
0.4
0.2
0

Fig. 3. Average linkage among 256 individuals in 983 Geneland MCMC replicates.

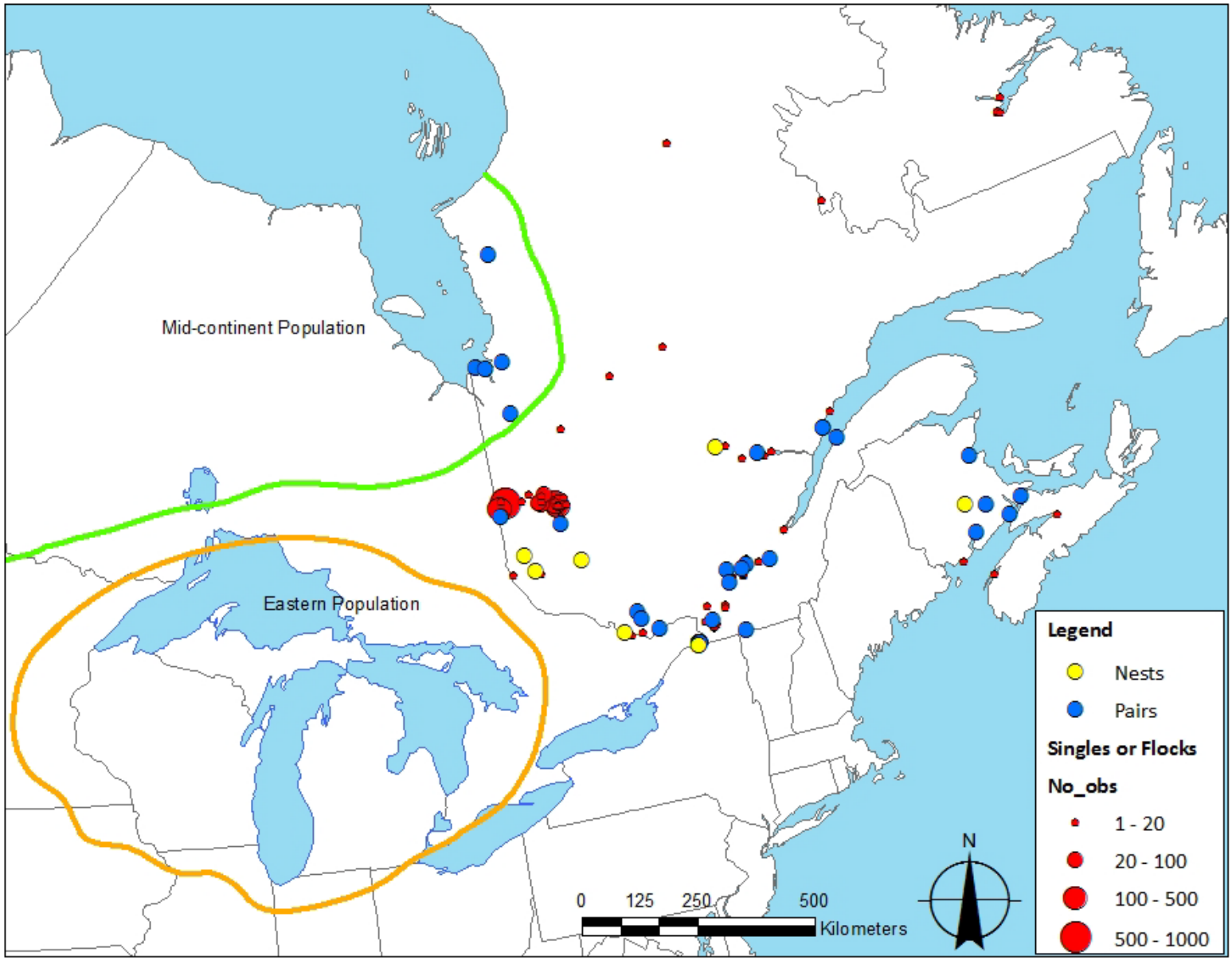

Fig. 4. Nests and observations of SACRs in eastern Canada from 1972 – 2012.

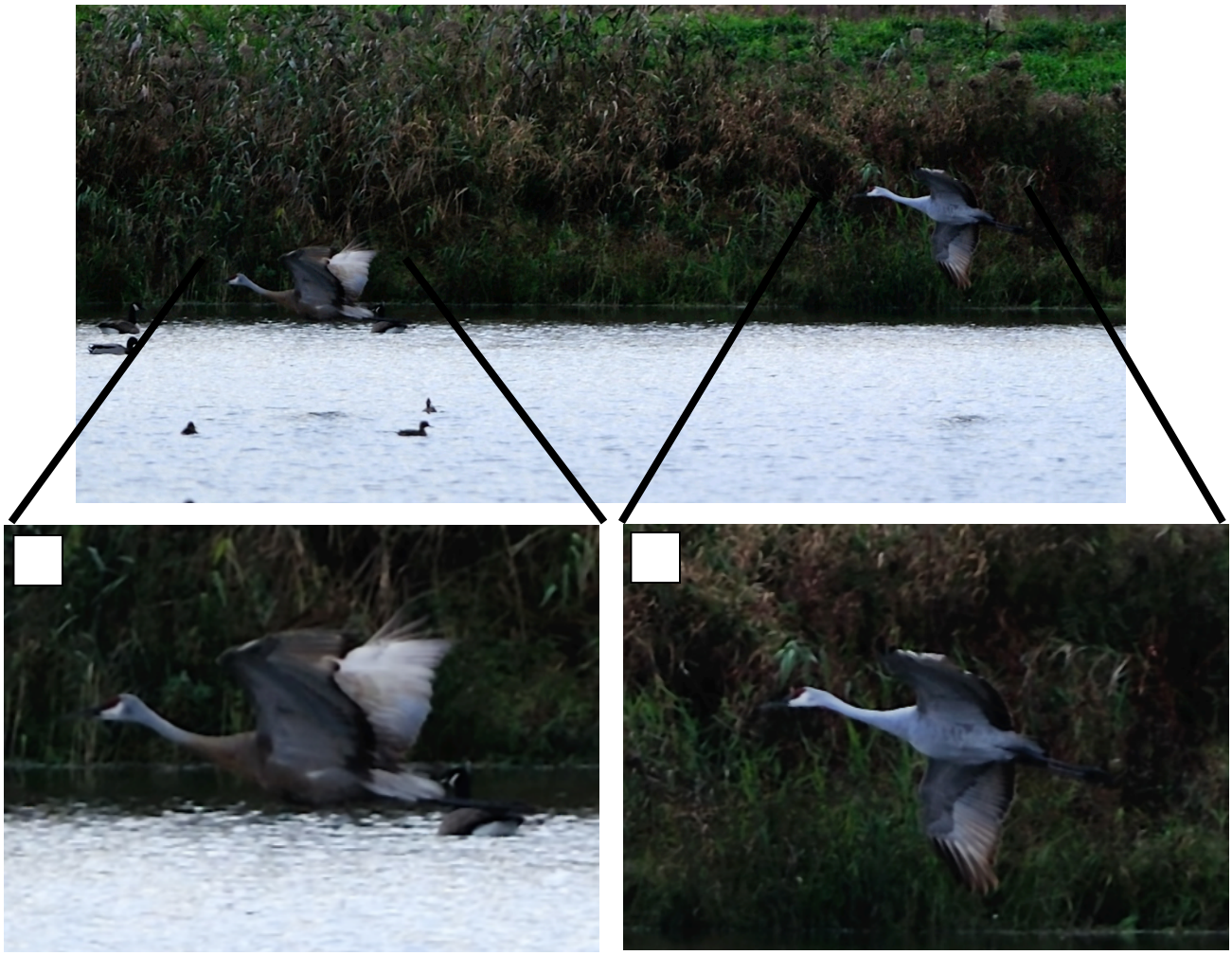

Fig. 5. Photograph of SACRs taken at Montezuma National Wildlife Refuge, NY on October 28, 2011 by Douglas Racine. (a) The "brown" bird is larger with relatively long bill and wings compared to overall body size suggesting *G. c. tabida*. (b) The "gray" bird is smaller with shorter bill and wings compared to overall body size which could suggest *G. c. rowani*.