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Published in:
Entomologia Experimentalis et Applicata

DOI:
10.1111/j.1570-7458.2009.00881.x

Publication date:
2009

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Female dispersal and isolation-by-distance of *Nasonia vitripennis* populations in a local mate competition context

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Accepted: 7 May 2009

Key words: mark-release-recapture, microsatellites, *Nasonia giraulti,* parasitoid, sex ratio, LMC, Hymenoptera, Chalcidoidea, Pteromalidae, jewel wasp

Abstract

Dispersal behavior directly influences the level of inbreeding, but the effect of inbreeding avoidance on dispersal is less well studied. The parasitoid wasp *Nasonia vitripennis* (Walker) (Hymenoptera: Chalcidoidea: Pteromalidae) is known to mate exclusively on the natal patch, and females are the only dispersing sex. A previous study has shown that foundresses on a patch are typically unrelated, implying that females disperse for a considerable distance from their natal patch after mating. We investigated dispersal of *N. vitripennis* on two scales. On a local scale we used a mark-release-recapture experiment, and on the larger scale we investigated isolation by distance using a population genetic approach. We found that *N. vitripennis* females are long-distance dispersers, capable of covering at least 2 km in 48 h. Populations within a range of 100 km showed no substructure, but larger distances or major geographical barriers restricted gene flow and led to significant population structure. The results provide a basis for future research on dispersal of parasitoids and are discussed in the context of dispersal abilities and inbreeding avoidance in *Nasonia*.

Introduction

The essence of local mate competition (LMC) theory is that a female should adjust the sex ratio of her offspring in such a way that competition between relatives is minimized (Hamilton, 1967). This is an evolutionarily stable strategy if females are the only dispersing sex, and if mating takes place exclusively at the natal patch. The most extreme case is that a patch population is founded by a single female. In this scenario, all males are brothers and the best strategy for the ovipositing female (here called foundress) would be to shift the sex ratio towards more daughters to reduce the competition among her sons. In a scenario with multiple foundresses, the outcome of LMC strongly depends on the relatedness among them. If the foundresses are unrelated, the sons of the different families compete with each other to mate with the available daughters. Therefore, for an individual foundress it is beneficial to produce a higher proportion of sons. In this scenario, the patch sex ratio will approach 0.5 when the number of foundresses per patch becomes very large (Fisher, 1930). In contrast, related foundresses produce related offspring, which results in the maintenance of the high level of LMC for a female's sons and a weaker LMC response is expected (Frank, 1985; Herre, 1985).

The jewel wasp, *Nasonia vitripennis* (Walker) (Hymenoptera: Chalcidoidea: Pteromalidae), is a gregarious parasitoid of cyclorraphous flies that is mainly found in bird nests. As its life history closely resembles the assumptions of LMC theory, it has been used extensively in LMC research (Werren, 1984; Drapeau & Werren, 1999; Shuker et al., 2004, 2006a, b). Laboratory experiments and three field studies (Werren, 1984; Molbo & Parker, 1996; Burton-Chellew et al., 2008) showed that *N. vitripennis* modulates its progeny sex ratio largely according to LMC theory.

The strict local mating of *N. vitripennis* strongly enhances inbreeding. Inbreeding combined with genetic drift leads to a loss of genetic variation and increased homozygosity, collectively termed as genetic erosion. In diploid organisms, this process can lead to the expression

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of homozygous recessive deleterious alleles. As Hymenoptera are haplodiploid, the effect of inbreeding is thought to be limited due to purging of such deleterious alleles in haploid males (Werren, 1993). However, one study on *N. vitripennis* (Luna & Hawkins, 2004) and one on *Uscana semifumipennis* Girault (Henter, 2003) found evidence that there is some inbreeding depression in parasitoids, as outcrossed strains performed better than the original inbred field strains. Another effect of the loss of genetic variation is the reduced ability to react to a changing environment (Bijlsma & Loeschcke, 2005). Therefore, purging of deleterious alleles in males is not sufficient to avoid the consequences of the loss of variation, as the adaptive potential is dependent on the amount of available genetic variation.

These theoretical considerations lead us to seek for mechanisms of inbreeding avoidance in *Nasonia*. A field study on *N. vitripennis* found slightly lower inbreeding than expected under random mating among the offspring of unrelated females in a patch (population inbreeding coefficient $F_{IT} = 0.17$, compared with an expected value of 0.22 under the assumption of unrelated females parasitizing a patch; Grillenberger et al., 2008). The assumption that all foundresses are unrelated requires a large well-mixed population. The usual behavior of a *Nasonia* female is to disperse right after mating and search for new hosts (Whiting, 1967; BK Grillenberger, pers. comm.). Although the primary purpose of female dispersal is the colonization of new host patches, also the composition of patches and the associated level of relatedness among the foundresses depend on the dispersal strategy of the mated females. Low dispersal distances could lead to a high level of relatedness and, vice versa, high dispersal distances to a low level of relatedness between foundresses within a patch. In *N. vitripennis* it has been shown that the foundress population of a patch is a random sample of the wasp population of the area (Grillenberger et al., 2008). This suggests that *N. vitripennis* females do leave their natal patch after mating and disperse a rather long distance. However, this study was confined to *N. vitripennis* in two rather small areas, and the actual dispersal distances have not yet been studied. In the present study, we use a mark-release-recapture experiment to estimate the dispersal capabilities of *N. vitripennis* and its sister species *Nasonia girault* (Darling) on a local scale. To estimate dispersal capabilities on a larger scale, we also performed a population genetic analysis on *N. vitripennis* samples.

Materials and methods

Mark-release-recapture experiment

To avoid the effects of changes in behavior due to long laboratory culturing, we used recently collected strains for the mark-release-recapture experiment. For *N. girault* we used strain NGVA collected in summer 2006 in Giles County (37°20′N, 80°46′W, VA, USA). For *N. vitripennis* we used either emerging individuals from freshly collected bird nests (species identity was based on male offspring emerging from a single host), or we used strain ITH4c which was collected in summer 2006 in Ithaca (42°30′N, 76°28′W, NY, USA). The 2006 strains have been in laboratory culture for about 20 generations. All strains were cultured on *Sarcophaga bullata* Parker (Diptera: Sarcophagidae) hosts at room temperature, until 1 day after emergence, and then kept at 4 °C until release.

Cornell University (Ithaca, NY, USA) allowed us to use its large array of bird nest boxes to perform a mark-release-recapture experiment (Figure 1). At this field site, nest boxes are mounted on approximately 1-m-high poles around either small ponds (western part) or in a large shallow pond (east side). For our experiment, we used all nest boxes along a transect from north to south (maximum distance = 445 m) and along a second transect from east to west (maximum distance = 415 m). At the intersection of these transects we released the marked wasps (~95% females) inside an empty nest box. For recapture, we placed mesh bait bags with 20 host pupae (*S. bullata*) inside all (34) nest boxes along the transects. Most nest boxes were empty but, if not, we placed the bait under the existing nest material as that is where natural hosts are usually found (BK Grillenberger, pers. comm.).

All wasps were counted while transferring them to a new culture vial (100 wasps per vial) and stained with fluorescent dust the evening before the release, by adding a small amount of dust to the vial and rolling the vial until all wasps were covered. The wasps were then kept at room temperature (20–25°C) until their release the next morning to give them the opportunity to clean off the excess dust. A previous test in the laboratory had shown that the wasps are able to clean off most of the dust, except at the base of the wings, where the fluorescent dust could be easily detected after 1 week of maintenance at 25 °C. On the release day, we first placed the baits for recapture in the appropriate nest boxes. Around 10:00 hours, the culture vials with the stained wasps were placed inside the release nest box and opened. Every 24 h, we collected all baits and replaced them with fresh ones, until the end of each release experiment. All collected baits were checked under UV light for traces of fluorescent dust, as well as for any *Nasonia* present on the hosts. The baits were kept at room temperature until either flies or wasps emerged to check whether hosts had been parasitized without a wasp being detected on the bait. For consecutive releases, the dust colors were changed to be able to assign recaptured individuals to a certain release date, because multiple releases were

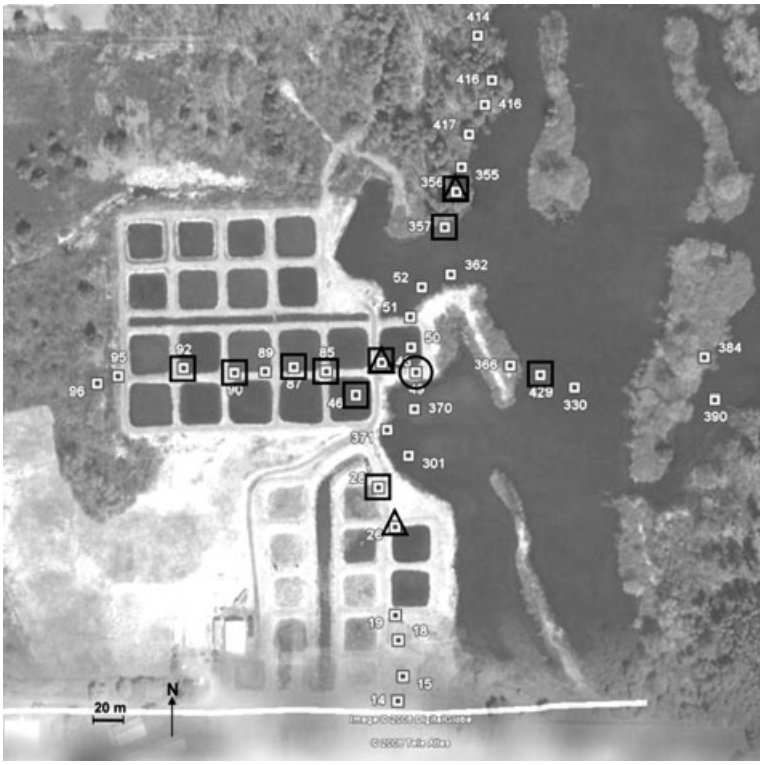

Figure 1 Approximate position of the nest boxes used in the mark-release-recapture experiment close to Ithaca, NY, USA (42°30′N, 76°28′W). Nest box 49 was used as release site (circle). In boxes labeled with a black square we found unmarked wasps, in boxes labeled with a black triangle we recaptured marked wasps. Map drawn with Google Earth.

done in the area during the season. Before a consecutive release, all remaining wasps (mostly males) were removed from the release nest box.

Molecular analysis

In order to evaluate the population genetic consequences of dispersal on a larger scale for *N. vitripennis*, we genetically analyzed samples from North America and Europe. For North America we used samples from two locations in Ithaca, one in Brewerton (NY), and three in Utah; for Europe we used samples from two locations on Corsica and nine localities across central Europe. Ten samples per area/population were examined (with central Europe as one area), using one individual per sampling location (mostly a nest box) to avoid artifacts due to relatedness between the samples of one nest box (for a complete sample list see Appendix 1).

We used eight polymorphic microsatellites to estimate the genetic differentiation between the study populations (LW Beukeboom, O Niehuis, BA Pannebakker, T Koevoets, JD Gibson, DM Shuker, L van de Zande, J Gadau, unpubl.; Table 2). PCR was performed using the Qiagen Multiplex kit (Qiagen, CA, USA) and fragment lengths were determined on an ABI 3730XL sequencer (Perkin-Elmer Applied Biosystems, CA, USA).

The software package F-stat (Goudet, 2001) was used to calculate Nei's G_{ST} values as well as Weir & Cockerham's F_{ST} at different hierarchical levels within and between North America and Europe. Hedrick's G'_{ST} (Hedrick, 2005), which is adjusted for marker variation, was calculated by hand on the basis of the F-stat output. As G_{ST} values tend to be slightly negative due to rounding errors, negative G_{ST} values were set to zero for the isolation by distance analysis, and the calculation of G'_{ST} (Meirmans, 2006). An isolation by distance analysis was performed by correlating the logarithm of geographical distance with G'_{ST}, using linear regression analysis in R (R Development Core Team, 2006). We compared these data with that of a previous study (Grillenberger et al., 2008) that calculated F_{ST} and G'_{ST} values in *N. vitripennis* as well.

Results

Mark-release-recapture experiment

A total of five releases were performed (three times only *N. vitripennis*, once only *N. giraulti*, once both species). The time between release and last collection of the recapture baits was 1–3 days. The time between subsequent releases was 2–6 days. After 24 h, we exclusively recaptured marked males in the release nest box; all marked

females had dispersed. Out of 3 150 released *N. vitripennis* specimens only three marked females were recaptured within the study area (20 m East, 120 m North, and 100 m South of the release point). In the same period and area, we caught 31 unmarked females in the baited nest boxes (Figure 1). Assuming an equal probability of catching marked and unmarked individuals, this leads by extrapolation to a population in the study area of around 27 000 individuals. The low recapture rate does not allow any conclusions about dispersal patterns within the area. However, we recaptured two marked *N. vitripennis* females in a nearby study area in which we conducted a different experiment. This area is about 2 km east of the release site. This indicates that individual *N. vitripennis* females can cover a distance of at least 2 km within 2 days. An onsite weather station recorded primarily westerly winds during the study period, suggesting that the wasps have been carried by wind currents. Most baits on which female wasps were collected also yielded wasp offspring, whereas the baits without signs of wasp presence did not. None of the 1 650 released *N. giraulti* were recaptured (Table 1).

Molecular analysis

In *N. vitripennis* the observed amount of genetic variation of the eight microsatellite markers was equal for the European and North American continent (Table 2). Between the sampling locations in New York, that were 2 and 100 km apart, no significant level of genetic differentiation was found (mean $F_{ST} = -0.01$). However, we did find a considerable amount of differentiation between the two locations in New York and a population from Utah (ca. 3 000 km apart) with a mean $F_{ST} = 0.10$. Between the European mainland and Corsica we found a level of differentiation equal to that between Utah and New York. Differentiation between the two continents is only slightly higher (see Table 3 for all F_{ST} values and geographic distances).

The isolation by distance analysis showed a significant correlation between genetic and geographic distance (linear regression: adjusted $R^2 = 0.679$, $F_{1,4} = 11.58$, $P = 0.027$). A comparison with the data of Grillenberger et al. (2008) on genetic differentiation of European populations shows good consistency between both studies (Table 3).

Discussion

The main goal of this paper was to find out how dispersal might influence population-level amounts of inbreeding, using both a local experimental approach and a more global population genetic approach.

Although the data from the recapture experiment are limited, they demonstrate that *N. vitripennis* females are able to disperse at least over a distance of 2 km. As the wasps were released in high densities, the effect of the release method on dispersal has to be considered. The usual number of wasps emerging from a single host is around 20, and there are up to several tens of hosts parasitized in a single nest (Grillenberger et al., 2009; BK Grillenberger, unpubl.). Because hosts are typically available for parasitation during a short period, several hundreds of wasps emerging from a single nest within a short time span is not uncommon in nature. Therefore we consider it unlikely that the release situation triggered unusual dispersal behavior. The wind records from an onsite weather station showed mostly westerly winds during the experiment, so the dispersal direction can be explained by wind drift. Small parasitoids are part of the aerial plankton and totally dependent on wind drift for dispersal, but for larger species, very little is known (Godfray, 1994; Quicke, 1997). Long-distance dispersal with the help of wind currents seems to be common among fig wasps and distances of several tens of kilometers, even over open sea, are no major obstacle for dispersal (Harrison, 2003; Zavodna et al., 2005). The parasitoid *Anagrus delicatus* Dozier has also been found to disperse over several kilometres (Antolin & Strong, 1987). Although most tiny species such as fig wasps and *A. delicates*, seem to be mainly transported by wind,

Table 1 Numbers and timing of five mark-release-recapture studies with *Nasonia vitripennis* (NV) and *N. giraulti* (NG) in Ithaca, NY, USA

Release	No. NV	No. NG	Colors used	Release date (2007)	Last collection date (2007)	Unmarked NV	Marked NV
1	300	0	Yellow	27 June	28 June	0	0
2	500	0	Blue	2 July	4 July	2	2
3	0	800	Green	5 July	7 July	5	0
4	850	850	Blue/ yellow	7 July	10 July	23	1
5	1 500	0	Green	12 July	14 July	1	0[1]
Total	3 150	1 650				31	3

There were no *N. giraulti* recaptured.

[1]Two *N. vitripennis* were recaptured at 2 km distance.

Table 2 Markers used, the number of alleles and expected heterozygosity (H_T; Nei, 1987), in North American (NA) and European (EU) samples of *Nasonia vitripennis*

Marker	No. alleles NA	H_T NA	No. alleles EU	H_T EU	No. alleles total	GenBank accession #
NV104	11	0.872	9	0.891	12	FJ156110
NV109	14	0.779	14	0.920	20	FJ156114
NV111	16	0.909	15	0.947	25	FJ156115
NV114	15	0.724	11	0.913	20	FJ156231
NV300	4	0.552	3	0.464	4	FJ156211
NV308	9	0.643	5	0.800	11	FJ555533
NV313	13	0.862	9	0.711	13	FJ156221
NV316	5	0.680	6	0.730	7	FJ156228
Average	10.88	0.753	9	0.797	14	

Table 3 Pairwise F_{ST} (after Cockerham & Weir, 1993), P-value following G-statistics over all loci (as implemented in F-stat), Hedrick's G'_{ST} (Hedrick, 2005), and approximate geographic distance between *Nasonia vitripennis* populations within North America and Europe

Sampling groups	F_{ST}	P	G'_{ST}	Approximate distance (km)
IthacaU1 – IthacaU2	−0.025	0.675	0	2
IthacaU1 – Brewerton	0.002	0.267	0.006	100
IthacaU2 – Brewerton	−0.013	0.233	0	100
IthacaU2 – Utah	0.089	0.008[1]	0.302	3 000
IthacaU1 – Utah	0.083	0.016	0.317	3 000
Brewerton – Utah	0.111	0.008[1]	0.406	3 000
Total NY – Utah	0.103	0.05[1]	0.357	3 000
EU mainland – Corsica	0.185	0.05[1]	0.613	500
Total EU – North America	0.133	0.05[1]	0.552	5 000
Within EU[2]	0.035		0.23	300

[1]Significant at nominal level of 0.05 after Bonferroni-correction for multiple comparisons.
[2]Data from Grillenberger et al. (2008).

Leptopilina heterotoma (Thomson) has been found to preferably fly against the wind (Papaj & Vet, 1990). A study on *Cotesia flavipes* Cameron (Sallam et al., 2001) showed a rather high recapture rate (6.7%) in a 100 × 100 m plot, and a pronounced decline in recapture numbers towards the edges of the plot, indicating a low dispersal distance. Several studies on *Trichogramma* spp. (see Kuske et al., 2003) found dispersal distances to a maximum of 400 m from the release site, which was also wind assisted. This indicates that there are clear differences between parasitoid species in their dispersal behavior. There seem to be two general strategies: (1) short-distance dispersal with directed movement, and (2) long-distance dispersal with the help of wind and presumably followed by directed movement within a close range.

As expected, the *N. vitripennis* males that were released together with the females did not disperse and could still be found at the release site. *Nasonia vitripennis* males have very short wings and are incapable of flight (Darling & Werren, 1990). The evidence for long-distance dispersal of *N. vitripennis* females is in line with the low genetic differentiation between the New York locations, where we found that gene flow is still possible within a range of 100 km. Also, given the high numbers of offspring emerging from a single host (ca. 20; Grillenberger et al., 2009), high gene flow over relative long distances is conceivable (Zavodna et al., 2005).

We did not recapture any released *N. giraulti*. This could be attributed to the lower number of individuals released, or the use of laboratory hosts as bait. *Nasonia giraulti* is believed to be a specialist of *Protocalliphora* fly pupae (Darling & Werren, 1990), but the baits were filled with *Sarcophaga* pupae. Hence, it is conceivable that *N. giraulti* was not as attracted to the baits as the generalist *N. vitripennis*. Another reason could be the differences in flight capability. Lehmann & Heymann (2006) showed that *N. vitripennis* females are just able to hover in mid air, but their maximal flight performance does hardly exceed that level. However, *N. giraulti* seems to be able to produce more lift with its wings and might, hence, be a better flyer and have left the study area completely.

The intercontinental comparison represents a maximum level for the F_{ST} as there is no gene flow between the continents. High mutation rate in microsatellites bears the risk of homoplasy and the measured level of differentiation of long diverged lineages could as a result be masked due to saturation in variation (Nauta & Weising, 1996). However, the observed high level of differentiation in the intercontinental comparison indicates that homoplasy is not an issue with the markers used in our study. The genetic differentiation between the island population on Corsica and the European mainland, as well as between Utah and New York, indicates that large bodies of water and mountain ranges are impassable barriers for *Nasonia*, as expected.

Taken together, our data suggest that *N. vitripennis* is a long-distance disperser that uses wind currents, resulting in high levels of gene flow across distances of 100 km. The implications for applying LMC theory to *Nasonia* are that the founding population of a local patch can be considered a random sample originating from a large area.

For a parasitoid such as *Nasonia*, that is exclusively dependent on a patchily distributed host, dispersal is the only way to find new opportunities for reproduction. In the release experiment, a large number of suitable hosts (ca.

8% of the baits have been parasitized by naturally occurring unmarked wasps) were presented within a close range, but most of the released wasps did not use them, and recapture rates were low. Although we cannot exclude that our laboratory cultured wasps were less efficient in finding hosts, a more satisfying explanation is that females initially disperse a large distance to avoid inbreeding. This can be interpreted as an adaptive strategy to minimize the level of genetic erosion given the characteristics of the life cycle of *N. vitripennis*. As already mentioned in the introduction, a high level of dispersal and the admixture of a large population lead to the maintenance of a large genetic variability that enables the population to react to stresses in a variable environment. Burton-Chellew et al. (2008) found that relatedness among the co-foundresses parasitizing a patch has no influence on the produced sex ratio, which is in contrast to LMC theory (Frank, 1985, 1998; Taylor & Crespi, 1994; Greeff, 1996; Reece et al., 2004). However, if *N. vitripennis* generally disperse over long distances, the chance that closely related females meet each other in a patch is likely to be negligible. As such, there would be little selection on the recognition of close relatives among co-foundresses.

To give a more detailed answer to the question how far *Nasonia* females disperse after mating, a more refined study covering distances as large as 1 000 km for a population genetic approach and about 2 km for a release experiment would be advisable. We believe that the insights obtained from the present study will provide valuable background information to conduct such a study.

Acknowledgments

We thank Saleta Perez-Vila, Kirsten Jalvingh, and Francisco Encinas-Viso for help in the field, John H. Werren and Rachel Edwards for providing us with wasps and hosts, Adèle Mennerat for providing samples from Corsica, and David Winkler and John Rogers for giving us access to their nest boxes. This work was supported by a field grant of the Schure-Beijerinck-Popping Fonds of the Royal Netherlands Academy of Sciences and Pioneer Grant ALW833.02.003 of the Netherlands Organization for Scientific Research to L.W.B.

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Appendix 1 List of *Nasonia* samples, with location and sampling group, used for the population structure analysis

Sample ID	Location	Sampling group
Cor01	Pirio, Corsica	Cor
Cor02	Pirio, Corsica	Cor
Cor03	Pirio, Corsica	Cor
Cor04	Pirio, Corsica	Cor
Cor05	Muro, Corsica	Cor
Cor06	Muro, Corsica	Cor
Cor07	Muro, Corsica	Cor
Cor08	Muro, Corsica	Cor
Cor09	Muro, Corsica	Cor
Cor10	Pirio, Corsica	Cor
Bay1	Bayreuth (Bavaria), Germany	EU
HH01	Hamburg, Germany	EU
HH03	Hamburg, Germany	EU
S004	Schlüchtern (Hessen), Germany	EU
ITA1	Montevarchi (Toscana), Italy	EU
AWD1	Amsterdam, The Netherlands	EU
BU2000-2	Bussum, The Netherlands	EU
FIG1	Valence, France	EU
SPA1	Spanderswoud, The Netherlands	EU
CH02	Oftringen, Switzerland	EU
MON013	Huntsville, Utah	UT
MON014	Huntsville, Utah	UT
MON011	Huntsville, Utah	UT
MON010	Huntsville, Utah	UT
MON012	Huntsville, Utah	UT
MON015	Huntsville, Utah	UT
PE020	Ogden, Utah	UT
SL010	Provo, Utah	UT
SL012	Provo, Utah	UT
SL011	Provo, Utah	UT
A252	Brewerton, New York	Brew
A476	Brewerton, New York	Brew
A396	Brewerton, New York	Brew
A342	Brewerton, New York	Brew
A363	Brewerton, New York	Brew
A055	Brewerton, New York	Brew
A091	Brewerton, New York	Brew
A605	Brewerton, New York	Brew
A271	Brewerton, New York	Brew
A652	Brewerton, New York	Brew
A509	Brewerton, New York	Brew
A379	Brewerton, New York	Brew
A255	Brewerton, New York	Brew
A580	Brewerton, New York	Brew
A341	Brewerton, New York	Brew
A235	Ithaca, New York	U1
A238	Ithaca, New York	U1
A318	Ithaca, New York	U1
A336	Ithaca, New York	U1
B105	Ithaca, New York	U1

Appendix 1 Continued

Sample ID	Location	Sampling group
B110	Ithaca, New York	U1
B115	Ithaca, New York	U1
B128	Ithaca, New York	U1
B142	Ithaca, New York	U1
B155	Ithaca, New York	U1
B300	Ithaca, New York	U1
B311	Ithaca, New York	U1
B317	Ithaca, New York	U1
B731	Ithaca, New York	U1
A083	Ithaca, New York	U2
A086	Ithaca, New York	U2
A197	Ithaca, New York	U2
A227	Ithaca, New York	U2
A247	Ithaca, New York	U2
B103	Ithaca, New York	U2
B109	Ithaca, New York	U2
B113	Ithaca, New York	U2
B114	Ithaca, New York	U2
B145	Ithaca, New York	U2
B191	Ithaca, New York	U2

Cor = Corsica, EU = European mainland, UT = Utah, Brew = Brewerton, U1 = IthacaU1, and U2 = IthacaU2.