

Development of a *Nasonia vitripennis* outbred laboratory population for genetic analysis

LOUIS VAN DE ZANDE,* STEVEN FERBER,* AMMERINS DE HAAN,* LEO W. BEUKEBOOM,* JOOST VAN HEERWAARDEN† and BART A. PANNEBAKKER*‡

*Evolutionary Genetics, Centre for Ecological and Evolutionary Studies, University of Groningen, 9700 CC Groningen, the Netherlands, †Biometris, Wageningen University, 6708 PB Wageningen, the Netherlands, ‡Laboratory of Genetics, Wageningen University, 6708 PB Wageningen, the Netherlands

Abstract

The parasitoid wasp genus *Nasonia* has rapidly become a genetic model system for developmental and evolutionary biology. The release of its genome sequence led to the development of high-resolution genomic tools, for both interspecific and intraspecific research, which has resulted in great advances in understanding *Nasonia* biology. To further advance the utility of *Nasonia vitripennis* as a genetic model system and to be able to fully exploit the advantages of its fully sequenced and annotated genome, we developed a genetically variable and well-characterized experimental population. In this study, we describe the establishment of the genetically diverse HVRx laboratory population from strains collected from the field in the Netherlands. We established a maintenance method that retains genetic variation over generations of culturing in the laboratory. As a characterization of its genetic composition, we provide data on the standing genetic variation and estimate the effective population size (N_e) by microsatellite analysis. A genome-wide description of polymorphism is provided through pooled resequencing, which yielded 417 331 high-quality SNPs spanning all five *Nasonia* chromosomes. The HVRx population and its characterization are freely available as a community resource for investigators seeking to elucidate the genetic basis of complex trait variation using the *Nasonia* model system.

Keywords: effective population size, genetic variation, laboratory strain, parasitoid wasp, pooled resequencing, single-nucleotide polymorphism (SNP)

Received 7 August 2013; revision received 30 October 2013; accepted 6 November 2013

Introduction

The parasitoid wasp genus *Nasonia* has rapidly become a genetic model system for developmental and evolutionary biology (Desplan & Beukeboom 2003; Werren & Loehlin 2009; Brown 2010; Godfray 2010; Muers 2010). As a genetic model, it has inherent advantages that rival even the current model insect systems with a much longer history and tradition. Its haplodiploid reproduction offers advantages of haploid genetics (e.g. lack of genetic dominance) in a complex eukaryotic system (Pultz *et al.* 2000). Several other important characteristics (easy husbandry, visible mutation markers, four infertile species, short-generation time and large family sizes, long-term storage after diapause induction) have laid the foundations for the large-scale study of *Nasonia*. However, the most important step in the development of *Nasonia* as a genetic model has been the availability of its

genome sequence (Werren *et al.* 2010), which largely expanded the understanding of basic biological processes in this species complex.

Of all the members within the *Nasonia* genus, *Nasonia vitripennis* is the best studied species, for which a wealth of information has been available on life-history and behavioural traits (e.g. Wylie 1966; Legner & Gerling 1967; Whiting 1967; Smith & Pimentel 1969; Davies 1975; Werren 1980, 1983a,b; King 1993; Rivers & Denlinger 1995; Drapeau & Werren 1999; Gadau *et al.* 1999; Bordenstein *et al.* 2001; Rivero & West 2002, 2005; Beukeboom & van den Assem 2004; Shuker & West 2004; Lalonde 2005; Shuker *et al.* 2005, 2007a,b; Bordenstein & Werren 2007; Pannebakker *et al.* 2008; Gadau & Wolschin 2009; Geuverink *et al.* 2009; Werren & Loehlin 2009; Raychoudhury *et al.* 2010a; Desjardins *et al.* 2010; Rivers *et al.* 2012; Niehuis *et al.* 2013; Gibson *et al.* 2013; Brucker & Bordenstein 2013). The release of its genome sequence led to the development of high-resolution genomic tools, for both interspecific (Niehuis *et al.* 2010; Loehlin *et al.*

Correspondence: Bart A. Pannebakker, Fax: +31 (0) 317 418094; E-mail: bart.pannebakker@wur.nl

2010a; Loehlin & Werren 2012; Desjardins *et al.* 2013) and intraspecific (Beukeboom *et al.* 2010; Pannebakker *et al.* 2010; Wang *et al.* 2013) research. The last years have seen a large number of studies that used the *Nasonia* genome sequence, reporting scientific breakthroughs in fundamental and applied biology (Loehlin *et al.* 2010b; Pannebakker *et al.* 2010, 2011; Verhulst *et al.* 2010; Werren *et al.* 2010; Lynch *et al.* 2011; Koevoets *et al.* 2012; Loehlin & Werren 2012; Brucker & Bordenstein 2013; Gibson *et al.* 2013; Niehuis *et al.* 2013; Paolucci *et al.* 2013). However, to further advance the utility of *N. vitripennis* as a genetic model system and to be able to fully exploit the advantages of its fully sequenced and annotated genome, a genetically variable and well-characterized experimental population, which is available for use in research worldwide, is highly required (e.g. Burke *et al.* 2010; Huang *et al.* 2012; Svenson *et al.* 2012).

In this study, we describe the establishment of the genetically diverse outbred HVRx laboratory population from strains that were collected from birds nest boxes and carrion baits in the Netherlands. Furthermore, we determine its (population) genetic characteristics and establish a breeding method that retains genetic variation over generations. To characterize the HVRx population, we describe the standing genetic variation and effective population size by microsatellite analysis. Pooled resequencing further yielded 417 331 high-quality SNPs, which are used for a genome-wide, high-resolution characterization of the population polymorphism. The HVRx population and its characterization are freely available as a community resource for investigators seeking to elucidate the genetic basis of complex trait variation using the *Nasonia* model system, for instance as a stock for association mapping in experimental evolution studies.

Materials and methods

HVRx population establishment and maintenance

In 2001, five *Nasonia vitripennis* strains were collected from different localities within 20 km radius from longitude 5.3 E and latitude 52.1 N in the Netherlands: Elspeek (strain 'B5'), Bussum (strain 'Bussum') and the Hoge Veluwe (strains 'HV55', 'HV287' and 'HV295'). Strains from the Hoge Veluwe were collected from bird nest boxes, while those from Elspeek and Bussum were collected from baits. Baits consisted of mesh screen bags, containing 25 laboratory hosts (*Calliphora vicina*) and a piece of liver. Each field strain was initiated by collecting all emerging female wasps from a nest box or bait and providing these with hosts. The number of founding females was not known, but host patches are usually colonized by multiple foundresses (Grillenberger *et al.* 2009b). After four generations of culturing (hosting about

100 females), ten mated females were randomly collected from each strain and individually provided with two hosts. From the offspring of each mated female, three virgin females and one male were isolated and mixed to initiate the HV population. Thus, a total 30 virgin females and 10 males from each strain were mixed in a 250-mL culture bottle and allowed to mate randomly. After 24 h, 200 hosts were added and the wasps were placed at 25 °C, under 16:8 light:dark conditions. After emergence, the HV population was split into two replicate populations (HV1 and HV2), by randomly isolating 100 mated females for each replicate and providing them with 200 hosts per replicate. Both replicates were maintained at 15 °C, 16:8 light:dark conditions.

To maximize genetic diversity, we merged the HV1 and HV2 replicates to form one single HVRx population after approximately 36 generations of separate mass culturing. From both replicates, we hosted 80 mated females on 200 hosts, and the offspring was allowed to mate randomly before dividing the wasps over four mass culture tubes (70 × 20 mm) each containing 50 fly hosts for oviposition. After 1 week, the parasitized hosts were distributed over four new mass culture tubes. After emergence, approximately 30 mated females from each mass culture tube were transferred to new mass culture tubes to initiate the next generation (Fig. 1). This breeding procedure ensures the maintenance of a large outbred population by allowing random mating between wasps of different tubes and was designed to preserve genetic diversity across generations. The HVRx population was maintained on *C. vicina* pupae as hosts, at 25 °C, 16: 8 light:dark conditions.

Microsatellite analysis

To determine and monitor the genetic diversity of the HVRx population over time, we isolated DNA from females from the HV1 ($n = 14$) and HV2 ($n = 14$) strains, and from the HVRx population in generations 1 ($n = 12$), 5 ($n = 24$), 10 ($n = 24$) and 32 ($n = 24$), using a standard high-salt-chloroform protocol (Maniatis *et al.* 1982). A set of 40 microsatellite markers, evenly distributed over the five chromosomes of *N. vitripennis* and combined into six multiplex sets, was used to determine genetic variation (Beukeboom *et al.* 2010; Pannebakker *et al.* 2010; Koevoets *et al.* 2012). Details of these microsatellite markers are listed in Table S1 (Supporting information). Microsatellite markers were amplified using the Qiagen multiplex PCR kit (Qiagen, Hilden, Germany) according to the manufacturer's recommendations (PCR profile: 15 min at 95 °C, followed by 30 cycles of 30 s at 94 °C, 1.5 min at annealing temperature and 1 min at 72 °C, followed by 45 min at 72 °C). Amplification was in 5 μ L volumes using Applied Biosystems Veriti or Applied

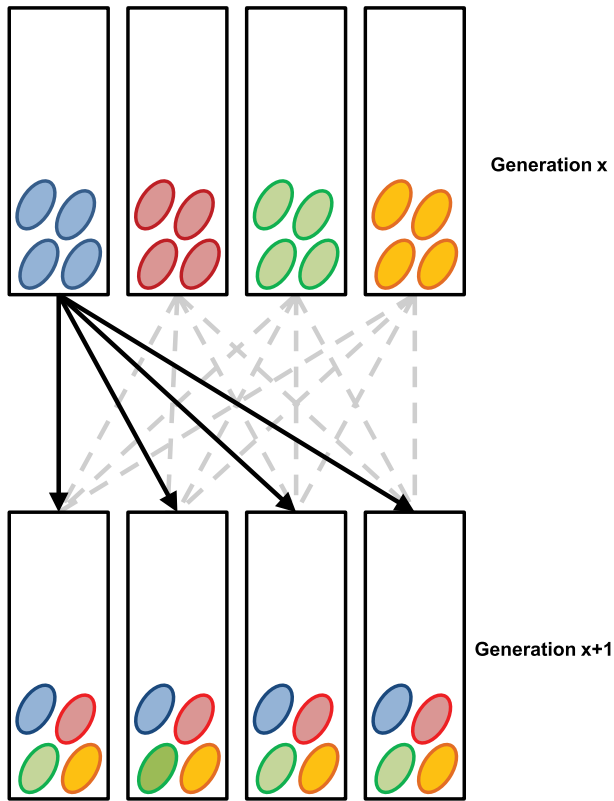


Fig. 1 *Nasonia vitripennis* HVRx outbred laboratory population maintenance schedule. In each of four mass culture tubes, 40 mated female *N. vitripennis* wasps of generation x are provided with 50 *Calliphora* spp. hosts. After oviposition, the parasitized hosts are redistributed over four clean mass culture tubes, to ensure optimal mixing of the wasps over all four culture tubes and to allow mating between all wasps emerging within generation $x + 1$.

Biosystems 9700 thermocyclers (Applied Biosystems, Foster City, CA, USA). Fragments were diluted 400 times, separated on a Applied Biosystems 3730 DNA Analyzer and analysed using GeneMapper v4.0 (Applied Biosystems). Population genetic statistics (allelic richness R , heterozygosity H_E , F_{ST}) were calculated using FSTAT 2.9.3 (Goudet 2001). Statistical analyses were carried out using R 2.14.1 (R Development Core Team 2012).

Theoretical estimate of effective population size

To evaluate the expected effective population size (N_e) achieved by our set-up, we simulated a population propagated under the four-tube breeding schedule that was used for maintenance of the HVRx population, as well as a population of identical census size but propagated as a single large one (i.e. in a single large tube). We modelled the sampling of a single biallelic locus in a haplodiploid system with 30 (four-replicates) or 120 (single-replicate) mated females per tube, to match the approximate

number of mated females transferred each generation. For each replicate, mating between diploid females and haploid males was simulated, with females producing offspring from a single male selected with replacement from a vector of males of length rN_f , where N_f is the number of females and r is the observed male over female ratio. For the multiple-replicate scheme, mated females from different tubes were mixed in equal proportions into new tubes (following Fig. 1).

For each simulation, we calculated F_t , the allelic differentiation with respect to the founding population, as: $F_t = \frac{(p_t - p_0)^2}{p_0(1 - p_0)}$ (Nicholson *et al.* 2002), where p_t is the allele frequency in generation t and p_0 is the frequency in the founding population. N_e was then estimated by solving the following nonlinear equation: $\bar{F}_t = 1 - \left(1 - \frac{1}{2N_e}\right)^t$, where \bar{F}_t was obtained by averaging F_t over 100 000 independent simulations. This simulated estimate of N_e was compared with the classic theoretical prediction of inbreeding effective size in a haplodiploid populations derived by Wright (1933) as:

$$\frac{9N_fN_m}{2N_f + 4N_m}$$

where N_f and N_m are the number of females and the number of males, respectively.

Empirical estimation of effective population size

We used approximate Bayesian computation (ABC, Tavaré *et al.* 1997; Marjoram *et al.* 2003) to obtain estimates of the posterior distribution of realized N_e over the past 32 generations from the microsatellite data. We estimated the following summary statistics from the data in generation 5, 10 and 32: number of alleles, expected heterozygosity and F_t (as defined above and averaged over all loci and alleles). This resulted in a vector of length 9, O , with each element O_{ij} containing the observed value for summary statistic i in generation j . We then simulated 10 000 000 instances of multilocus genotypes sampled from a diploid Wright–Fisher population subject to drift over 32 generations, with proposal values of N_e drawn from a uniform distribution between 0 and 1000. Starting allele frequencies were set to match those observed in the base population. In each independent simulation, the same summary statistics as above were measured in generation 5, 10 and 32, yielding a vector S of simulated summary statistics, with each element S_{ij} corresponding to a value in O_{ij} . Following Pritchard *et al.* (1999), we only accepted iterations for which $|S_{ij} - O_{ij}| / O_{ij} < \epsilon$ for each element of S_{ij} and O_{ij} . The threshold value ϵ was set to 0.11 to achieve an acceptance rate of 0.001.

Genome resequencing and analysis

To resequence the HVRx base population, DNA from 20 pooled females from generation four was extracted and purified using Qiagen DNeasy columns (Qiagen). A 101-bp unpaired and a 76-bp paired-end library were constructed and subsequently sequenced by the University Medical Centre Groningen (UMCG) Genome Analysis Facility according to standard Illumina protocols. The unpaired library was run on a single lane and the paired-end library on five lanes of an Illumina Genome Analyzer II (Illumina, San Diego, CA, USA). Sequence data were provided to NasoniaBase (Munoz-Torres *et al.* 2011) and submitted to the NCBI Short Read Archive with accession no. SRP022050.

Reads were aligned to the v2.0 of the *N. vitripennis* genome (Werren *et al.* 2010) using Mosaik Aligner v1.1.0021 (<https://code.google.com/p/mosaik-aligner/>). SNPs were filtered from the HVRx assembly using VARSCAN.v2.2.8 (Koboldt *et al.* 2012), according to the following rules: minimum consensus quality: 20; minimum count of minor allele: 2, minimum coverage: 8. Reads with mapping qualities less than 20 were discarded. SNP data were provided to NasoniaBase (Munoz-Torres *et al.* 2011) and deposited in Dryad (Dryad Entry doi:10.5061/dryad.f2330).

Mean SNP densities were calculated in nonoverlapping 100 kb windows, with a minimum mean coverage of 8. Genome-wide diversity analysis was performed using the popoolation pipeline (Kofler *et al.* 2011). We estimated nucleotide diversity π in nonoverlapping 100 kb windows, using the same parameters augmented with a maximum coverage of 1 000 000, a fraction of the window that has a coverage between the minimum and maximum coverage of 0.6. Additional statistical analyses were carried out using R 2.14.1 (R Development Core Team 2012).

Results

After being maintained in the laboratory for approximately 36 generations, the HV1 and HV2 populations

had genetically diverged ($F_{ST} = 0.26, P < 0.05$, Table 1). The mean heterozygosity was not statistically different between both replicates (mean $H_E \pm SE$ HV1 = 0.53 ± 0.03 , HV2 = 0.46 ± 0.05 , $t_{74} = 1.41, P = 0.163$, Table 2), which is reflected in the heterozygosity per locus for HV1 and HV2 (Table S2, Supporting information). The mean allelic richness did show differences between both replicate populations (mean $R \pm SE$ HV1 = 3.08 ± 0.18 , HV2 = 2.52 ± 0.14 , $t_{78} = 2.50, P = 0.015$, Table 2).

The HV1 and HV2 replicates were merged to start the HVRx population with maximum genetic diversity. We subsequently cultured the HVRx population following a breeding procedure designed to retain genetic diversity over generations (Fig. 1). To confirm the effectiveness of our breeding procedure, we monitored the population genetic characteristics of the HVRx population at generations 1, 5, 10 and 32 after establishment.

Genetic differentiation

Already at generation 1, the HVRx population had significantly diverged from both original populations ($F_{ST} = 0.04$ (HV1) and 0.16 (HV2), $P < 0.05$, Table 1). After generation 5, the HVRx population started to diverge modestly but significantly between subsequent generations (Table 1). However, 32 generations of controlled breeding resulted in a F_{ST} value of 0.07 between generation 1 and generation 32, which is considerably lower than that of the differentiation between the original HV1 and HV2 populations after 36 generations (Table 1).

Genetic variation

Mean heterozygosities ($H_E \pm SE$) for HVRx generations 1, 5, 10 and 32 did not change and were 0.58 ± 0.04 , 0.60 ± 0.03 , 0.56 ± 0.03 and 0.56 ± 0.03 , respectively. An overall comparison, including also the founding populations HV1 and HV2, showed that the only significant difference in heterozygosity was between HV2 and HVRx generation 5 (Tukey's HSD critical value = 0.127, Table 2). Our results indicate that heterozygosity was

Table 1 Genetic differentiation in the *Nasonia vitripennis* HVRx outbred laboratory population. Pairwise F_{ST} values between HV1 and HV2 founder populations and generations 1, 5, 10 and 32 of the HVRx population over 40 microsatellite loci combined

	HV1	HV2	HVRx-G1	HVRx-G5	HVRx-G10	HVRx-G32
HV1	0.00	0.26*	0.04*	0.04*	0.06*	0.11*
HV2		0.00	0.16*	0.17*	0.18*	0.22*
HVRx-G1			0.00	0.02	0.03*	0.07*
HVRx-G5				0.00	0.03*	0.06*
HVRx-G10					0.00	0.05*
HVRx-G32						0.00

*Significant values $P < 0.05$ after Bonferroni correction following G-statistics (as implemented in Fstat).

Table 2 Genetic variation in the *Nasonia vitripennis* HVRx outbred laboratory population

	Expected heterozygosity H_E	Allelic richness R
HV1	0.53 (0.03) ^a	3.08 (0.18) ^{ab}
HV2	0.46 (0.04) ^{ab}	2.52 (0.14) ^a
HVRx-G1	0.58 (0.04) ^a	3.52 (0.22) ^b
HVRx-G5	0.60 (0.03) ^{ac}	3.45 (0.20) ^b
HVRx-G10	0.56 (0.03) ^a	3.40 (0.18) ^b
HVRx-G32	0.56 (0.03) ^a	3.34 (0.21) ^b

Table shows the mean expected heterozygosity H_E and the mean allelic richness R . Standard errors are in parentheses.

Different lowercase letters indicate significant differences at $P < 0.05$ for each estimate.

effectively maintained over 32 generations. This pattern is also reflected in the per-locus heterozygosity (Table S2, Supporting information).

Mean allelic richness ($R \pm SE$) showed a slight decrease over generations 1, 5, 10 and 32 and was 3.52 ± 0.22 , 3.45 ± 0.20 , 3.40 ± 0.18 and 3.34 ± 0.21 , respectively. An overall comparison, including also the founding populations HV1 and HV2 only, showed significant differences in allelic richness between HV2 and all HVRx generations (One-way AOV; $F_{5,234} = 3.87$, $P = 2.170e-3$; Tukey HSD critical value = 0.741, Table 2). Overall, our results indicate that our controlled breeding method effectively limits the decrease in allelic richness over 32 generations.

Effective population size

The empirical estimation of effective population size (N_e), based on the diversity parameters above, yielded a posterior mode of 195 and a mean of 236 diploid individuals. The computer simulations of the different breeding schemes yielded nearly identical effective population sizes of $N_e = 177$ and $N_e = 173$ for the four-replicate and single-replicate mating scheme, respectively, entirely consistent with the theoretical prediction of $N_e = 173$ and falling within 0.9 and 0.3 standard deviations of the posterior mean and mode, respectively (Fig. 2).

Genomic variation

We found considerable genetic variability in the HVRx population, with a total of 417 331 SNPs distributed over the five chromosomes, or about 1 SNP per 796.7 bp [based on a physical genome size of 332.5 Mb (Gregory, 2013)]. SNP density varied between the chromosomes (one-way AOV; $F_{4,1880} = 9.58$, $P = 1.16e-07$, Table 3), with the lowest mean density per 100 kb window on chromosome 1. The pattern in SNP density differentiation is

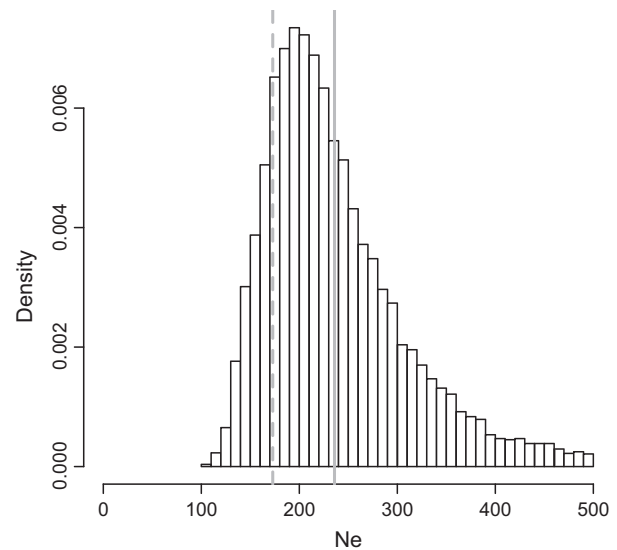


Fig. 2 Effective population size N_e in the *Nasonia vitripennis* HVRx outbred laboratory population. Posterior density of the empirical estimate of N_e based on 40 microsatellite loci, obtained by approximate Bayesian computation. Blue, red and green lines indicate the theoretical prediction based on Wright (1933), the posterior mean and posterior median of the empirical estimate, respectively.

retained (one-way AOV; $F_{4,1880} = 1105.1$, $P < 2.2e-16$, Table 3, Fig. 3) upon adjusting for differences in read depth between the different chromosomes (one-way AOV; $F_{4,1880} = 12.268$, $P = 7.55e-10$, Table 3, Fig. S1, Supporting information). Interestingly, the highest SNP density per 100 kb window was also found on chromosome 1 at 25.3 Mb showing 438 SNPs (or 39.84 SNPs after adjusting for 10.99X read depth).

The mean nucleotide diversity per 100 kb window over all chromosomes was 0.13%, showing significant differentiation by chromosome (one-way AOV; $F_{4,155} = 9.97$, $P = 5.76e-08$, Table 3), with chromosome 1 showing the lowest nucleotide diversity compared with other chromosomes. The distribution of genomic variation over the chromosomes showed similar patterns for both SNP density as for nucleotide diversity. Both measures were higher towards the tips of the chromosomes than the centres (Figs 3 and 4). Estimates of nucleotide diversity were lacking in areas where the read depths were low, such as the near the centre of the chromosomes (Fig. 4).

Discussion

The HVRx laboratory population captures a large amount of genetic variation. With over 400 000 SNPs, it is a valuable resource for investigating the genetic architecture of complex trait variation in the *Nasonia* genetic model system. The HVRx population provides

Table 3 Genomic variation per chromosome in the *Nasonia vitripennis* HVRx outbred laboratory population. Mean SNP density, read depth adjusted SNP density, read depth and nucleotide diversity per chromosome in 100 kb nonoverlapping sliding windows

	Mean SNP density in 100 kb windows (SE)	Mean read depth adjusted SNP density in 100 kb windows (SE)	Mean read depth per 100 kb window (SE)	Mean nucleotide diversity (π) in 100 kb windows (SE)
1	179.57 (5.83) ^a	3.96 (0.13) ^a	45.05 (0.71) ^a	0.0011 (4.00e-05) ^a
2	219.10 (5.74) ^{bc}	4.43 (0.10) ^{bc}	49.32 (0.64) ^b	0.0014 (3.54e-05) ^b
3	214.99 (6.44) ^{bcd}	4.53 (0.13) ^{bcd}	47.95 (0.79) ^{bc}	0.0013 (4.30e-05) ^b
4	197.96 (6.62) ^{acd}	4.21 (0.12) ^{acd}	46.90 (0.70) ^{ac}	0.0013 (4.54e-05) ^b
5	224.59 (6.34) ^b	4.37 (0.12) ^b	51.07 (0.70) ^b	0.0014 (3.85e-05) ^b
All	202.29 (2.80)	4.28 (0.06)	47.84 (0.32)	0.0013 (1.83e-05)

Standard errors are in parentheses. Different lowercase letters indicate significant differences at $P < 0.05$ for each estimate.

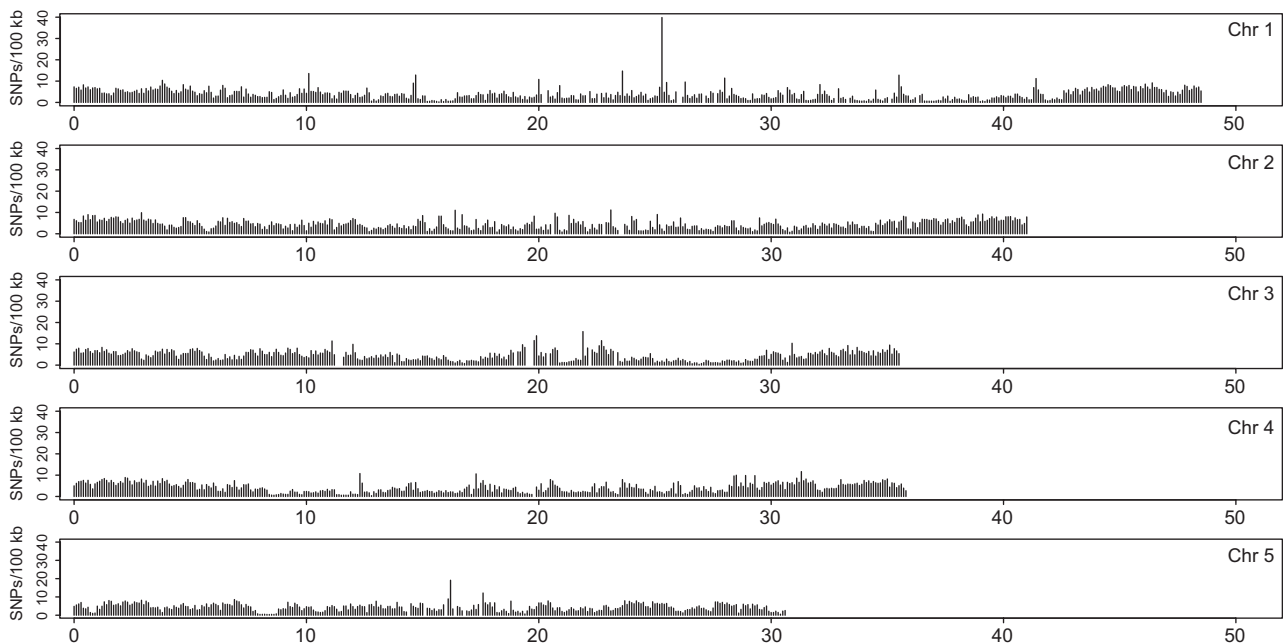


Fig. 3 Genome-wide SNP density pattern in the *Nasonia vitripennis* HVRx outbred laboratory population. Mean SNP density adjusted for read depth in nonoverlapping 100 kb windows, plotted against chromosomal position.

a genetically variable stock population for experimental evolution experiments, in which the availability of genomic information enables efficient association mapping of complex traits. While genetically diverse stock populations are available for other model species (*Drosophila*: Burke *et al.* 2010; Huang *et al.* 2012; mouse: Svenson *et al.* 2012; *Arabidopsis*: Kover *et al.* 2009), HVRx is the first available for the Hymenoptera genetic model *Nasonia*.

The HVRx population is maintained at a breeding schedule that retains the available genetic variation, evidenced by the fact that over 32 generations, both the heterozygosity H_E and the allelic richness R had not changed significantly. While there was greater differentiation between both founding HV replicates ($F_{ST} = 0.26$)

than between European and North American *Nasonia vitripennis* populations ($F_{ST} = 0.15$, Raychoudhury *et al.* 2010a), our breeding schedule effectively prevented further differentiation over generations (Table 1). Using a simple breeding procedure, our set-up results in a simulated population size of $N = 177$, and an effective population size N_e of 236. This equals to an N_e/N ratio close to 1.5, which is high compared with previously reported estimates for natural and captive populations of other species (Briscoe *et al.* 1992; Frankham 1995; Simoes *et al.* 2010). While effective population size is influenced by many factors, such as fluctuations in population size over generations, variation in family size and sex ratio, mating system and selection, work by Simoes *et al.* (2010) has shown that careful maintenance procedures

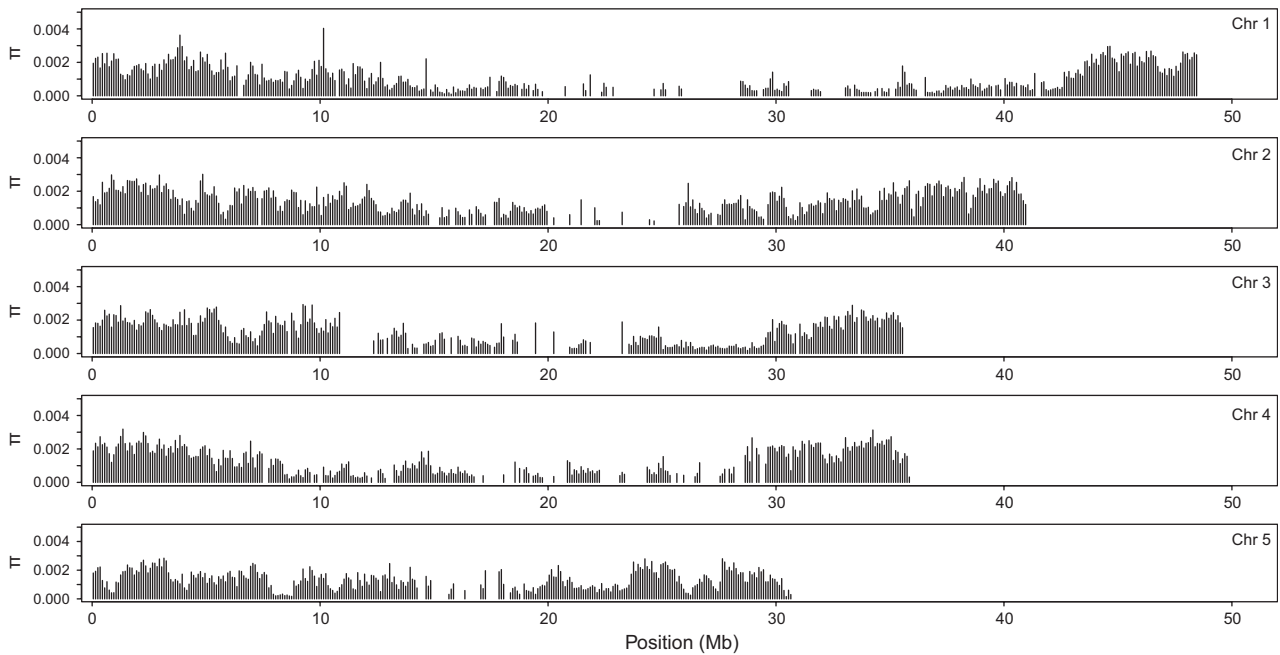


Fig. 4 Genome-wide polymorphism pattern in the *Nasonia vitripennis* HVRx outbred laboratory population. Mean estimates of nucleotide diversity (π) in nonoverlapping 100 kb windows, plotted against chromosomal position.

can efficiently reduce the loss of genetic variability in laboratory populations in organisms that generate a high number of offspring. The results described here for the HVRx population corroborate this finding.

The effective population size is an important parameter for predicting the potential response to selection (Falconer & Mackay 1996). Because the effect of drift is reduced in larger populations, allelic diversity persists longer allowing for greater and faster responses to selection (Weber 2004). While the exact response to selection is determined by intensity of selection, heritability of the trait of interest and effective population size, the empirical estimate of $N_e = 236$ for HVRx is on upper range used in artificial selection studies (Weber & Diggins 1990; Weber 2004; Zhang & Hill 2005). The population sizes of laboratory populations also received attention in the biological control literature, where the maintenance of genetic variability is an important issue. The population size of the HVRx exceeds the general recommendation to start and maintain natural enemy cultures with an effective population size of $N_e > 100$ (Roush 1990; Bartlett 1993; Nunney 2003).

The genetic diversity of the HVRx laboratory population is slightly lower than that observed in studies of natural *N. vitripennis* populations. Evaluating 12 overlapping microsatellite loci, the heterozygosity of the HVRx population after 32 generations (mean 0.52 ± 0.07) is lower than that observed in natural *N. vitripennis* populations directly upon collection from the field (range of H_E : 0.46–0.95 Table S3, Supporting information;

Grillenberger *et al.* 2009a; Raychoudhury *et al.* 2010b; Paolucci *et al.* 2013). It is, however, considerably higher than in the highly inbred standard laboratory strain AsymCX (Werren *et al.* 2010). A similar pattern is observed in the genome-wide nucleotide diversity of the HVRx population ($\pi = 0.0013$), which is lower than the levels of synonymous nucleotide diversity (ranging from $\pi = 0.0016$ to 0.0026) observed in ~16 kb sequence from 27 genes in multiple strains of *N. vitripennis* (Raychoudhury *et al.* 2010b; Werren *et al.* 2010). The genome-wide nucleotide diversity of the HVRx population, however, included both synonymous and nonsynonymous polymorphisms, which result in a lower estimate than when just synonymous polymorphisms were used. Overall, the levels of nucleotide diversity in *Nasonia* are much lower than those observed in natural *Drosophila melanogaster* populations (e.g. Mackay *et al.* 2012; Orozco-terWengel *et al.* 2012), which could be explained by founder events, but also by effects more specific to haplodiploids such as the purging of deleterious mutations in haploid males or high levels of inbreeding (Hedrick & Parker 1997).

Analysis of the genomic variation in the HVRx population shows that genomic polymorphism, both in terms of SNP density as well as of nucleotide diversity, is lower in the centre of the chromosomes and higher towards the distal ends. While we have no exact data on the location of the centromeres, the fact that all *Nasonia* chromosomes are meta- or submetacentric (Gokhman & Westendorff 2000) strongly suggests the centromeres are at or near

the centre of the chromosomes. These regions not only show reduced nucleotide diversity in the HVRx population, but also show reduced recombination rates in *Nasonia* (Niehuis *et al.* 2010; Desjardins *et al.* 2013), consistent with the correlation between recombination rate and nucleotide diversity observed in a wide range of organisms (Begun & Aquadro 1992; Nachman 2002). Unfortunately, the nucleotide diversity data are not complete throughout the HVRx genome, due to low read depths at several regions. While we cannot exclude sequence quality issues for these regions, low read depth regions collocate with gaps in the Nvit 2.0 reference sequence (Werren *et al.* 2010) that make reference mapping impossible in those regions.

The HVRx laboratory population is a genetically diverse *N. vitripennis* outbred laboratory population that fills a gap between conventional inbred *Nasonia* laboratory strains and free-living natural populations. It offers higher levels genetic variation than inbred laboratory strains and easier experimental manipulation than free-living populations, by capturing intraspecific genetic variation in a tractable laboratory population that is easily maintained using the provided breeding schedule. While inbred laboratory strains are a valuable tool for studying interspecific differences in *Nasonia* (e.g. Desjardins *et al.* 2010; Werren *et al.* 2010; Koevoets *et al.* 2012; Loehlin & Werren 2012; Gibson *et al.* 2013; Niehuis *et al.* 2013), the intraspecific context is crucial to understand the evolutionary forces underlying adaptation of complex (life-history) traits. The HVRx adds a novel resource for both inter- and intraspecific research in *Nasonia*, thereby further extending the qualities of the *Nasonia* genetic model system for developmental and evolutionary research. The combination of a genetically diverse outbred population with full genomic information makes HVRx a solid starting point for studying the genomic changes underlying the evolution of complex traits, for instance as a stock population for experimental evolution in combination with association mapping studies.

Acknowledgements

We thank Albert Kamping and Tim Hollander for their help in setting up the HV and HVRx populations, and Jürgen Gadau for sharing unpublished microsatellite primers with us. The research was supported by the Netherlands Genomics Initiative grants to BAP (NGI Horizon Breakthrough no. 935.19.006 and NGI Zenith no. 935.11.04) and by the Netherlands Organisation for Scientific Research NWO Grant No. ALW 833.02.003 to LWB.

References

Bartlett AC (1993) Maintaining genetic diversity in laboratory colonies of parasites and predators. In: *Applications of Genetics to Arthropods of*

- Biological Control Significance* (eds Narang S, Bartlett A, Faust R), pp. 133–145. CRC Press, Boca Raton, FL.
- Begun DJ, Aquadro C (1992) Levels of naturally occurring DNA polymorphism correlate with recombination rates in *D. melanogaster*. *Nature*, **356**, 519–520.
- Beukeboom LW, van den Assem J (2004) A review of *Nasonia* (Chalcidoidea, Pteromalidae) courtship and mating behaviour, with some additional new observations. *Proceedings of the section Experimental and Applied Entomology of the Netherlands Entomological Society*, **15**, 123–132.
- Beukeboom LW, Niehuis O, Pannebakker BA *et al.* (2010) A comparison of recombination frequencies in intraspecific versus interspecific mapping populations of *Nasonia*. *Heredity*, **104**, 302–309.
- Bordenstein SR, Werren JH (2007) Bidirectional incompatibility among divergent *Wolbachia* and incompatibility level differences among closely related *Wolbachia* in *Nasonia*. *Heredity*, **99**, 278–287.
- Bordenstein SRR, O'Hara FPP, Werren JH (2001) *Wolbachia*-induced incompatibility precedes other hybrid incompatibilities in *Nasonia*. *Nature*, **409**, 707–710.
- Briscoe DA, Malpica JM, Robertson A *et al.* (1992) Rapid loss of genetic variation in large captive populations of *Drosophila* flies: implications for the genetic management of captive populations. *Conservation Biology*, **6**, 416–425.
- Brown S (2010) The *Nasonia* genome sequence: finding gems in the jewel (wasp) box. *Insect Molecular Biology*, **19**, v–vii.
- Brucker RM, Bordenstein SR (2013) The hologenomic basis of speciation: gut bacteria cause hybrid lethality in the genus *Nasonia*. *Science*, **341**, 667–669.
- Burke MK, Dunham JP, Shahrestani P *et al.* (2010) Genome-wide analysis of a long-term evolution experiment with *Drosophila*. *Nature*, **467**, 587–590.
- Davies I (1975) A study of the effect of diet on the life-span of *Nasonia vitripennis* (Walk.) (Hymenoptera, Pteromalidae). *Journal of Gerontology*, **30**, 294–298.
- Desjardins CA, Perfectti F, Bartos JD, Enders LS, Werren JH (2010) The genetic basis of interspecies host preference differences in the model parasitoid *Nasonia*. *Heredity*, **104**, 270–277.
- Desjardins CA, Gadau J, Lopez JA *et al.* (2013) Fine-scale mapping of the *Nasonia* genome to chromosomes using a high-density genotyping microarray. *G3 (Bethesda)*, **3**, 205–215.
- Desplan C, Beukeboom LW (2003) *Nasonia*. *Current Biology*, **13**, R860.
- Drapeau MD, Werren JH (1999) Differences in mating behaviour and sex ratio between three sibling species of *Nasonia*. *Evolutionary Ecology Research*, **1**, 223–234.
- Falconer DS, Mackay TFC (1996) *Introduction to Quantitative Genetics*. Longmans Green, Harlow, Essex, UK.
- Frankham R (1995) Conservation genetics. *Annual Review of Genetics*, **29**, 305–327.
- Gadau J, Wolschin F (2009) Deciphering proteomic signatures of early diapause in *Nasonia*. *PLoS ONE*, **4**, e6394.
- Gadau J, Page REE, Werren JH *et al.* (1999) Mapping of hybrid incompatibility loci in *Nasonia*. *Genetics*, **153**, 1731–1741.
- Geuverink E, Gerritsma S, Pannebakker BA, Beukeboom LW (2009) A role for sexual conflict in the evolution of reproductive traits in *Nasonia* wasps? *Animal Biology*, **59**, 417–434.
- Gibson JD, Niehuis O, Peirson BRE, Cash EI, Gadau J (2013) Genetic and developmental basis of F2 hybrid breakdown in *Nasonia* parasitoid wasps. *Evolution*, **67**, 2124–2132.
- Godfray HCJ (2010) *Nasonia*: a jewel among wasps. *Heredity*, **104**, 235–236.
- Gokhman VE, Westendorff M (2000) The chromosomes of three species of the *Nasonia* complex (Hymenoptera, Pteromalidae). *Beiträge zur Entomologie*, **50**, 193–198.
- Goudet J (2001) *FSTAT*, a program to estimate and test gene diversities and fixation indices. Available from <http://www2.unil.ch/popgen/softwares/fstat.htm>.
- Gregory TR (2013) *Animal Genome Size Database*. Available from <http://www.genomesize.com>.

- Grillenberger BK, Gadau J, Bijlsma R, van de Zande L, Beukeboom LW (2009a) Female dispersal and isolation-by-distance of *Nasonia vitripennis* populations in a local mate competition context. *Entomologia Experimentalis et Applicata*, **132**, 147–154.
- Grillenberger BK, van de Zande L, Bijlsma R, Gadau J, Beukeboom LW (2009b) Reproductive strategies under multiparasitism in natural populations of the parasitoid wasp *Nasonia* (Hymenoptera). *Journal of Evolutionary Biology*, **22**, 460–470.
- Hedrick PW, Parker JD (1997) Evolutionary genetics and genetic variation of haplodiploids and X-linked genes. *Annual Review of Ecology and Systematics*, **28**, 55–83.
- Huang W, Richards S, Carbone MA *et al.* (2012) Epistasis dominates the genetic architecture of *Drosophila* quantitative traits. *Proceedings of the National Academy of Sciences of the United States of America*, **109**, 15553–15559.
- King BH (1993) Flight activity in the parasitoid wasp *Nasonia vitripennis* (Hymenoptera, Pteromalidae). *Journal of Insect Behavior*, **6**, 313–321.
- Koboldt DC, Zhang Q, Larson DE *et al.* (2012) VarScan 2: somatic mutation and copy number alteration discovery in cancer by exome sequencing. *Genome Research*, **22**, 568–576.
- Koevoets T, Niehuis O, van de Zande L, Beukeboom LW (2012) Hybrid incompatibilities in the parasitic wasp genus *Nasonia*: negative effects of hemizygoty and the identification of transmission ratio distortion loci. *Heredity*, **108**, 302–311.
- Kofler R, Orozco-terWengel P, De Maio N *et al.* (2011) PoPoolation: a toolbox for population genetic analysis of next generation sequencing data from pooled individuals. (M Kayser, Ed.) *PLoS ONE*, **6**, e15925.
- Kover PX, Valdar W, Trakalo J *et al.* (2009) A multiparent advanced generation inter-cross to fine-map quantitative traits in *Arabidopsis thaliana*. *PLoS Genetics*, **5**, e1000551.
- Lalonde RG (2005) Egg size variation does not affect offspring performance under intraspecific competition in *Nasonia vitripennis*, a gregarious parasitoid. *Journal of Animal Ecology*, **74**, 630–635.
- Legner E, Gerling D (1967) Host-feeding and oviposition on *Musca domestica* by *Spalangia cameroni*, *Nasonia vitripennis*, and *Muscidifurax raptor* (Hymenoptera: Pteromalidae) influences their longevity and fecundity. *Annals of the Entomological Society of America*, **60**, 678–691.
- Loehlin DW, Werren JH (2012) Evolution of shape by multiple regulatory changes to a growth gene. *Science*, **335**, 943–947.
- Loehlin DW, Enders LS, Werren JH (2010a) Evolution of sex-specific wing shape at the widerwing locus in four species of *Nasonia*. *Heredity*, **104**, 260–269.
- Loehlin DW, Oliveira DCSG, Edwards R *et al.* (2010b) Non-coding changes cause sex-specific wing size differences between closely related species of *Nasonia*. *PLoS Genetics*, **6**, e1000821.
- Lynch JA, Ozüak O, Khila A *et al.* (2011) The phylogenetic origin of oskar coincided with the origin of maternally provisioned germ plasm and pole cells at the base of the Holometabola. *PLoS genetics*, **7**, e1002029.
- Mackay TFC, Richards S, Stone EA *et al.* (2012) The *Drosophila melanogaster* Genetic Reference Panel. *Nature*, **482**, 173–178.
- Maniatis T, Fritsch EF, Sambrook J (1982) *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory, New York.
- Marjoram P, Molitor J, Plagnol V, Tavaré S (2003) Markov chain Monte Carlo without likelihoods. *Proceedings of the National Academy of Sciences of the United States of America*, **100**, 15324–15328.
- Muers M (2010) Evo-devo: *Nasonia* tools up. *Nature Reviews Genetics*, **11**, 170–171.
- Munoz-Torres MC, Reese JT, Childers CP *et al.* (2011) Hymenoptera Genome Database: integrated community resources for insect species of the order Hymenoptera. *Nucleic Acids Research*, **39**, D658–D662.
- Nachman M (2002) Variation in recombination rate across the genome: evidence and implications. *Current Opinion in Genetics & Development*, **12**, 657–663.
- Nicholson G, Smith AV, Jonsson F *et al.* (2002) Assessing population differentiation and isolation from single-nucleotide polymorphism data. *Journal of the Royal Statistical Society Series B-Statistical Methodology*, **64**, 695–715.
- Niehuis O, Gibson JD, Rosenberg MS *et al.* (2010) Recombination and its impact on the genome of the haplodiploid parasitoid wasp *Nasonia*. *PLoS ONE*, **5**, e8597.
- Niehuis O, Buellesbach J, Gibson JD *et al.* (2013) Behavioural and genetic analyses of *Nasonia* shed light on the evolution of sex pheromones. *Nature*, **494**, 7–10.
- Nunney L (2003) Managing captive populations for release: a population genetic perspective. In: *Quality Control and Production of Biological Control Agents: Theory and Testing Procedures* (ed. van Lenteren JC), pp. 73–97. CAB International, Wallingford, UK.
- Orozco-terWengel P, Kapun M, Nolte V *et al.* (2012) Adaptation of *Drosophila* to a novel laboratory environment reveals temporally heterogeneous trajectories of selected alleles. *Molecular Ecology*, **21**, 4931–4941.
- Pannebakker BA, Halligan DLDL, Reynolds KTT *et al.* (2008) Effects of spontaneous mutation accumulation on sex ratio traits in a parasitoid wasp. *Evolution*, **62**, 1921–1935.
- Pannebakker BA, Niehuis O, Hedley A, Gadau J, Shuker DM (2010) The distribution of microsatellites in the *Nasonia* parasitoid wasp genome. *Insect Molecular Biology*, **19**, 91–98.
- Pannebakker BA, Watt R, Knott SA, West SA, Shuker DM (2011) The quantitative genetic basis of sex ratio variation in *Nasonia vitripennis*: a QTL study. *Journal of Evolutionary Biology*, **24**, 12–22.
- Paolucci S, van de Zande L, Beukeboom LW (2013) Adaptive latitudinal cline of photoperiodic diapause induction in the parasitoid *Nasonia vitripennis* in Europe. *Journal of Evolutionary Biology*, **26**, 705–718.
- Pritchard JK, Seielstad MT, Perez-Lezaun A, Feldman MW (1999) Population growth of human Y chromosomes: a study of Y chromosome microsatellites. *Molecular Biology and Evolution*, **16**, 1791–1798.
- Pultz MA, Zimmerman KK, Alto NM *et al.* (2000) A genetic screen for zygotic embryonic lethal mutations affecting cuticular morphology in the wasp *Nasonia vitripennis*. *Genetics*, **154**, 1213–1229.
- R Development Core Team (2012) *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. Available from <http://www.R-project.org/>.
- Raychoudhury R, Desjardins CA, Buellesbach J *et al.* (2010a) Behavioral and genetic characteristics of a new species of *Nasonia*. *Heredity*, **104**, 278–288.
- Raychoudhury R, Grillenberger BK, Gadau J *et al.* (2010b) Phylogeography of *Nasonia vitripennis* (Hymenoptera) indicates a mitochondrial–Wolbachia sweep in North America. *Heredity*, **104**, 318–326.
- Rivero A, West SA (2002) The physiological costs of being small in a parasitic wasp. *Evolutionary Ecology Research*, **4**, 407–420.
- Rivero A, West SA (2005) The costs and benefits of host feeding in parasitoids. *Animal Behaviour*, **69**, 1293–1301.
- Rivers DB, Denlinger DL (1995) Fecundity and development of the ectoparasitic wasp *Nasonia vitripennis* are dependent on host quality. *Entomologia Experimentalis et Applicata*, **76**, 15–24.
- Rivers DBDB, Kaikis A, Bulanowski D, Wigand T, Brogan R (2012) Oviposition restraint and developmental alterations in the ectoparasitic wasp, *Nasonia vitripennis*, when utilizing puparia resulting from different size maggot masses of *Lucilia illustris*, *Protophormia terraenovae*, and *Sarcophaga bullata*. *Journal of Medical Entomology*, **49**, 1124–1136.
- Roush R (1990) Genetic variation in natural enemies: critical issues for colonization in biological control. In: *Critical Issues in Biological Control* (eds Mackauer M, Ehler L, Roland J), pp. 263–288. Intercept, Andover, UK.
- Shuker DM, West SA (2004) Information constraints and the precision of adaptation: sex ratio manipulation in wasps. *Proceedings of the National Academy of Sciences of the United States of America*, **101**, 10363–10367.
- Shuker DM, Pen I, Duncan AB, Reece SE, West SA (2005) Sex ratios under asymmetrical local mate competition: theory and a test with parasitoid wasps. *The American Naturalist*, **166**, 301–316.

- Shuker DM, Phillimore AJ, Burton-Chellew MN, Hodge SE, West SA (2007a) The quantitative genetic basis of polyandry in the parasitoid wasp, *Nasonia vitripennis*. *Heredity*, **98**, 69–73.
- Shuker DM, Reece SE, Lee A *et al.* (2007b) Information use in space and time: sex allocation behaviour in the parasitoid wasp *Nasonia vitripennis*. *Animal Behaviour*, **73**, 971–977.
- Simoes P, Pascual M, Coelho M, Matos M (2010) Divergent evolution of molecular markers during laboratory adaptation in *Drosophila subobscura*. *Genetica*, **138**, 999–1009.
- Smith GJC, Pimentel D (1969) The effect of two host species on the longevity and fertility of *Nasonia vitripennis*. *Annals of the Entomological Society of America*, **62**, 305–308.
- Svenson KL, Gatti DM, Valdar W *et al.* (2012) High-resolution genetic mapping using the Mouse Diversity outbred population. *Genetics*, **190**, 437–447.
- Tavare S, Balding D, Griffiths R, Donnelly P (1997) Inferring coalescence times from DNA sequence data. *Genetics*, **145**, 505–518.
- Verhulst EC, Beukeboom LW, van de Zande L (2010) Maternal control of haplodiploid sex determination in the wasp *Nasonia*. *Science*, **328**, 620–623.
- Wang X, Wheeler D, Avery A *et al.* (2013) Function and evolution of DNA methylation in *Nasonia vitripennis*. *PLoS genetics*, **9**, e1003872.
- Weber K (2004) Population size and long-term selection. (J Janick, Ed.) *Plant Breeding Reviews*, **24**, 249–268.
- Werren KE, Diggins LT (1990) Increased selection response in larger populations. II. Selection for ethanol vapor resistance in *Drosophila melanogaster* at two population sizes. *Genetics*, **125**, 585–597.
- Werren JH (1980) Sex ratio adaptations to local mate competition in a parasitic wasp. *Science*, **208**, 1157–1159.
- Werren JH (1983a) Sex ratio evolution under local mate competition in a parasitic wasp. *Evolution*, **37**, 116–124.
- Werren JH (1983b) Brood size and sex ratio regulation in the parasitic wasp *Nasonia vitripennis* (Walker) (Hymenoptera: Pteromalidae). *Netherlands Journal of Zoology*, **34**, 123–143.
- Werren JH, Loehlin DW (2009) The parasitoid wasp *Nasonia*: an emerging model system with haploid male genetics. *Cold Spring Harbor Protocols*, **10**, doi: 10.1101/pdb.emo134.
- Werren JH, Richards S, Desjardins CA *et al.* (2010) Functional and evolutionary insights from the genomes of three parasitoid *Nasonia* species. *Science*, **327**, 343–348.
- Whiting AR (1967) The biology of the parasitic wasp *Mormoniella vitripennis* (Walker). *Quarterly Review of Biology*, **42**, 333–406.
- Wright S (1933) Inbreeding and homozygosity. *Proceedings of the National Academy of Sciences of the United States of America*, **19**, 411–420.
- Wylie HG (1966) Survival and reproduction of *Nasonia vitripennis* (Walk) at different host population densities. *The Canadian Entomologist*, **98**, 275–281.
- Zhang X-S, Hill WG (2005) Predictions of patterns of response to artificial selection in lines derived from natural populations. *Genetics*, **169**, 411–425.

L.Z. and B.A.P. conceived and performed the research and wrote the paper. S.F. performed the microsatellite analyses. A.H. performed maintenance of HVRx and prepared the material for sequencing. L.W.B. collected the original field strains. J.H., L.Z. and B.A.P. performed statistical analyses.

Data Accessibility

Sequence data were provided to NasoniaBase (<http://hymenopteragenome.org/Nasonia/>) and submitted to the NCBI Short Read Archive with accession no. SRP022050. SNP data were provided to NasoniaBase and deposited in Dryad (Dryad Entry doi:10.5061/dryad.f2330).

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1 Read depth in the *N. vitripennis* HVRx outbred laboratory population.

Table S1 Microsatellite marker properties.

Table S2 Expected heterozygosities per microsatellite marker.

Table S3 Heterozygosity in HVRx generation 32 and natural *N. vitripennis* populations.