Ecological genomics of adaptation to unpredictability in experimental rotifer populations

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Elucidating the genetic basis of phenotypic variation in response to different environments is key to understanding how populations evolve. Facultatively sexual rotifers can develop adaptive responses to fluctuating environments. In a previous evolution experiment, diapause-related traits changed rapidly in response to two selective regimes (predictable vs unpredictable) in laboratory populations of the rotifer *Brachionus plicatilis*. Here, we investigate the genomic basis of adaptation to environmental unpredictability in these experimental populations. We identified and genotyped genome-wide polymorphisms in 169 clones from both selective regimes after seven cycles of selection using genotyping by sequencing (GBS). Additionally, we used GBS data from the 270 field clones from which the laboratory populations were established. This GBS dataset was used to identify candidate SNPs under selection. A total of 76 SNPs showed divergent selection, three of which are candidates for being under selection in the particular unpredictable fluctuation pattern studied. Most of the remaining SNPs showed strong signals of adaptation to laboratory conditions. Furthermore, a genotype-phenotype association approach revealed five SNPs associated with two key life-history traits in the adaptation to unpredictability. Our results contribute to elucidating the genomic basis for adaptation to unpredictable environments and lay the groundwork for future evolution studies in rotifers.

Unraveling the genetic mechanisms underlying organisms' phenotypic variation is essential to understanding how organisms adaptively respond to environmental fluctuations1–2. Anthropogenic effects and climate change have been identified as amplifiers of the variation in environmental fluctuations, and this effect is likely to further increase in the near future3. For that reason, adaptations to unpredictable fluctuations are particularly challenging to understand4–5. Research is needed to know whether organismal adaptive responses will allow them to cope with the upcoming increase in environmental unpredictability. Consequently, (1) understanding organisms' evolutionary responses to unpredictability and (2) unraveling the molecular mechanisms and the genomic basis underlying these adaptations are key topics in evolutionary and conservation biology6.

Various adaptive responses have been described in organisms that inhabit fluctuating environments4. One of them is phenotypic plasticity, in which the same genotype produces different phenotypes depending on the environmental conditions7. Another mode of evolutionary response is adaptive tracking, in which natural selection acts recurrently on the standing heritable variation8. A third adaptive response is bet hedging, a life-history strategy that has been linked to unpredictable fluctuations9. This response occurs when a selected trait in a genotype increases the geometric mean of fitness at the cost of a decrease in the arithmetic mean, thus reducing fitness variance10–12. Despite the relevance of the ubiquitous challenge posed by environmental fluctuations and the diversity of adaptive responses displayed by organisms, empirical studies elucidating the genomic basis of these responses are still scarce.

Experimental evolution is a powerful tool that can be used to address diverse questions in evolutionary biology13,14 and explore the genomic basis of adaptation15,16 because it involves controlled laboratory conditions with a defined selective pressure. Typically, in this type of experiment, populations derived from a single ancestral genotype—i.e., a genetically homogeneous population—are exposed to different selective regimes17,18. However, an increasing number of studies have used a combination of several genotypes from one or more populations—i.e., several ancestral genotypes or a polymorphic population—to establish the experimental populations19–20.
Evolutionary outcomes are expected to differ depending on whether the experimental populations are established from a single or from multiple genotypes. If the founding population is genetically monomorphic, adaptation to selective regimes could occur through the accumulation of de novo beneficial mutations. While not excluding this source of variation to fuel adaptive evolution, if the founding population is polymorphic—and given the relative short-term scale of evolution experiments—selection is expected to act mostly on the heritable standing variation21–23, which has been predicted to lead to rapid evolution in novel environments44.

Monogonont rotifers are common zooplankters of continental water bodies and are recognized as excellent model organisms for studies of experimental evolution (reviewed in Declerck & Papakostas25). These rotifers are facultatively sexual, combining asexual and sexual reproduction in their life cycle. Rotifer females reproduce asexually —clonal proliferation— until a specific environmental cue for sex initiation (e.g., the population density) induces some of these females to produce sexual daughters. These sexual females produce meiotic haploid eggs that develop into either haploid males, if they remain unfertilized, or into diploid diapausing eggs if they are fertilized26–28. Therefore, the product of sexual reproduction is a long-lived diapausing egg, so that sex is linked with diapause. Diapausing eggs are able to resist harsh environmental conditions such as desiccation, predation, freezing, etc.28–30. These eggs can remain viable in the sediments through at least several hydroperiods, forming diapausing egg banks, which are reservoirs of the past genetic diversity and adaptive phenotypic variation of the population31 and are essential for long-term population persistence32. In temperate climates, rotifer populations are typically ephemeral and recolonize the water column during the so-called planktonic growing season through the hatchlings of diapausing eggs, forming assemblages of clonal populations32–34. The rotifer species Brachionus plicatilis is a common inhabitant of salt lakes around the world35,36. These habitats are isolated from one another and are often characterized by strong seasonal fluctuations in the length of the growing season37 and can become unsuitable for periods of varying predictability38. These fluctuations are typical in the water bodies of the Mediterranean region39, which show a wide range of environmental predictability40. B. plicatilis populations in natural ponds are characterized by strong population structure and low levels of gene flow due to persistent founder effects35,41,42.

Using a set of nine B. plicatilis field populations from ponds covering a wide range of environmental predictability, Franch-Gras et al.43 identified several candidate single-nucleotide polymorphisms (SNPs) as part of the genomic basis of local adaptation to fluctuating environments, which constitute a database for future genomic studies45. In a previous evolution experiment, we used B. plicatilis to study the adaptation of life-history traits to unpredictable environments (for further details, see Tarazona et al.46). Six genetically identical laboratory populations were established by placing together 30 rotifer clones from each of the nine field populations representing a broad range of environmental unpredictability used by Franch-Gras et al.43. Since the rotifer clones from these populations were pooled to create the laboratory populations, hereafter we will refer to this set as the “origin population”. Three laboratory populations (functioning as replicates) were randomly assigned to each selective regime (predictable vs unpredictable). These populations were studied during seven cycles of selection under a common garden experimental approach, tracking the evolution of two key diapause-related life-history traits: (1) the timing of sex—as a proxy of the timing of diapause—and (2) the diapausing egg hatching fraction—inversely related to diapause duration. Rotifers showed rapid adaptation to a particular unpredictable fluctuation pattern of hydroperiod length, displaying a divergent response in those life-history traits in the populations subjected to the unpredictable regime with respect to those subjected to the predictable regime. Populations subjected to the unpredictable selective regime showed both lower hatching fractions of diapausing eggs and earlier sex initiation, suggesting that bet-hedging strategies evolve as an adaptation to environmental unpredictability in these organisms46. Although we used hydroperiod length fluctuation as the factor of unpredictability in our experimental design, it should be noted that several other factors (e.g., food, salinity, predation, etc.) might also affect the degree of predictability in the wild. Note that this experimental design involved testing for the effect of a particular pattern of unpredictable fluctuation in hydroperiod length, so we cannot generalize that the observed response is an adaptation to environmental unpredictability itself.

In this study, we used genotyping by sequencing (GBS)47 to identify the genomic signatures of selection. This technique relies on the use of restriction enzymes to reduce genome complexity and allows the identification and genotyping of single-nucleotide polymorphisms (SNPs) in individuals from different populations48,49. Thus, we identified and screened thousands of genomic markers by using GBS in laboratory B. plicatilis populations that evolved under predictable and unpredictable hydroperiod selective regimes48 to study the genomic basis of their adaptation. Additionally, to identify putative genes under selection and estimate the impact of genetic drift in the experimental populations, we used both an available B. plicatilis genome assembly from one clone from one of the field populations45 and genome-wide data obtained with the same methodology from the nine B. plicatilis field populations48 used to found the experimental laboratory populations. This large GBS dataset was used to identify candidate loci for diversifying selection under these selective regimes in the experimental rotifer populations. Moreover, the initial GBS information allowed us to estimate drift during the duration of the experiment for each particular laboratory population, as they can be compared with the origin population.
Results

GBS raw data and SNP calling and filtering. A total of 19 Gb of GBS raw data was obtained from the 169 clones from the six laboratory populations. A total of ca. 560 out of the ca. 915 million raw reads from the origin population plus the six laboratory populations were retained after quality filtering (see Material and Methods). After SNP calling and filtering, 6,107 SNPs were identified and genotyped (Table 1; vcf file: Supplementary Dataset S1) with an average coverage of 43.77 (7.02–109.25, median = 42.04). This set of SNPs was used in downstream analyses.

Genetic structure. Clones from the origin population —belonging to the nine field rotifer populations— were widespread in the PCA ordination space, whereas most of the six laboratory populations were clustered in the center (Fig. 1). Pairwise $F_{ST}$ values between the origin and the six laboratory populations were low, ranging from 0.004 to 0.025 (Table 2). These $F_{ST}$ values were low in comparison with those of the field populations.42,45
which suggests that genetic drift had little effect on the laboratory populations during the evolution experiment, although the unpredictable populations had slightly higher values, suggesting higher drift50,51. A mean FST value of 0.015 was found between selective regimes. Populations subjected to the predictable regime showed the lowest differentiation (Fig. 1 and Supplementary Fig. S1), with pairwise FST values ranging from 0.016 to 0.025 (Table 2). Populations under the unpredictable regime were more differentiated (Fig. 1 and Supplementary Fig. S1), with pairwise FST values ranging from 0.046 to 0.078. Expected heterozygosity (He) – i.e., genetic diversity – was maintained in all laboratory populations (ranging from 0.17 to 0.21) after the evolution experiment, except for one of the populations that evolved under the unpredictable regime (U2), which had the lowest genetic diversity (for further details, see Supplementary Table S1). The values of genetic diversity were similar to those in the origin population (He = 0.21). Overall, all populations analyzed were broadly in Hardy-Weinberg equilibrium (HWE; Supplementary Fig. S2).

Candidate SNPs under selection. A total of 76 candidate SNPs under selection were identified by BayeScan (BS) analysis with prior odds (PO) = 10 among the origin population and the two selective regimes (BS1 analysis; Fig. 2). These candidate SNPs were on 45 scaffolds showing evidence of being under selection. Three of the candidate SNPs under selection were identified in the comparison of the two selective regimes (BS2 analysis with PO = 10; red dots in Fig. 2; Supplementary Fig. S3). These three unlinked candidate SNPs (S4644_2726, S9060_3689 and S78024_5745) had undergone changes in allele frequencies in the populations under the unpredictable regime, diverging from the origin population (Table 3). This divergence was not found in the predictable populations, which had similar allelic frequencies to the origin population. Most of the remaining SNPs identified in the BS1 analysis showed parallel changes in all six laboratory populations, suggesting adaptation to laboratory conditions.

Genome-wide association analysis. A genotype-phenotype association analysis, using the subset of genotyped clones for which life-history traits were available, revealed a significant association between five SNPs and the assessed traits at a significance level of 0.05/N (N = 52 and 76 clones for the diapausing egg hatching fraction and the timing of sex, respectively). Four SNPs in two scaffolds (S1772_184, S27547_4262, S27547_4267 and S27547_4271) were associated with the hatching fraction (Fig. 3A and Supplementary Fig. S4), with an adjusted p-value = 0.0201 for SNP S1772_184 and p-value = 0.0015 for the other three SNPs. The three SNPs in
scaffold S27547 were 9 bases apart. One SNP (S5425_9210) was associated with the timing of sex (Fig. 3B and Supplementary Fig. S4) with an adjusted p-value = 0.0288.

Identification of candidate genes under selection. The 76 SNPs identified by BS1 as candidates of being under positive selection were located within 43 genes, 53.5% of which had putative associated functions (see Supplementary Table S2). Following Franch-Gras et al.'s criteria, additional analyses were performed using just those genes including a SNP in their coding region (i.e., genes at a 0 Kb distance). GO enrichment analysis

Figure 3. Genotype-phenotype association patterns for two life-history traits of B. plicatilis: hatching fraction and timing of sex. Box-and-whisker plot of the genotypes of SNPs that are most significantly associated with (A) diapausing egg hatching fraction (SNP S1772_184 and three SNPs associated to the scaffold S27547), and (B) timing of sex, estimated as the density for sex initiation (SNP S5425_9210). Dots (jittered in the x direction) represent each clone, those evolved under the predictable regime are represented by blue circles, whereas clones evolved under the unpredictable one by red triangles. Phenotypic data obtained from Tarazona et al.46.
showed that they were enriched at high levels (i.e., general GO terms), such as the regulation of biological processes (GO:0050789), cellular processes (GO:000987), single-organism processes (GO:0044699), the regulation of cellular processes (GO:0050794), binding (GO:0005488), and biological regulation (GO:0065007). The three candidate SNPs to be under selection between selective regimes were located in three genes: Ribosomal S6 kinase alpha-1 isoform X1, RNA-binding single-stranded-interacting 3 and Midasin.

Those SNPs identified as putatively associated with the hatching fraction of diapausing eggs in the genotype-phenotype analysis were located in two genes, but only one had an associated function: the gene kelch-like ECH-associated 1. No gene was found for the candidate SNP associated with the timing of sex.

Discussion

We identified genomic signatures underlying rapid adaptation in rotifer populations under two contrasting laboratory selective regimes (predictable vs unpredictable) using an experimental evolution approach and genome-wide genotyping. Three out of 45 scaffolds had signatures of divergent selection in response to an unpredictable environmental fluctuation pattern. Most of the remaining scaffolds with signatures of directional selection experienced parallel allele changes in all six laboratory populations, suggesting adaptation to laboratory environmental conditions. These populations showed signatures of adaptation to the experimental unpredictable environment in their life-history traits (i.e., the timing of sex and diapausing egg hatching fraction)46. In addition, the genome-wide association analysis performed here revealed three scaffolds associated with both life-history traits. Overall, our results indicate that there is substantial genetic diversity underlying the adaptation to environmental unpredictability in the original rotifer populations.

We found that three SNPs located in three different genes showed signatures of positive selection in response to the unpredictable environmental fluctuation pattern studied. The SNP S9060_3689 was located within the gene RNA-binding single-stranded-interacting 3 (RBMS3), a member of the family of c-myc single-strand binding protein (MSSP) genes. These genes are involved in DNA replication, gene transcription, cell cycle progression and apoptosis in humans52 and regulate one of the major pathways promoting chondrogenesis in zebrafish53. The SNP S4644_2726 is located within the ribosomal S6 kinase alpha-1 isoform X1 gene and is involved in controlling growth and differentiation in human cells54. Finally, the SNP S78024_5745 was located in the Midasin gene, which encodes a nuclear chaperone involved in the assembly/disassembly of complex macromolecules in yeast55 and has shown to be essential for the normal progression of female gametogenesis in Arabidopsis thaliana56. Regarding the genotype-phenotype association analysis, four SNPs putatively associated with the diapausing egg hatching fraction were found. Three of them were fully linked and located within the same gene—kelch-like ECH-associated 1—-which is involved in responses to oxidative stress57–59. This gene has been described in several groups, including fish60, insects61, and mammals62.

In general, the experimental populations showed weak genetic drift, as evidenced by the low genome-wide pairwise FST values (FST < 0.1; mean FST between selective regimes was 0.015) when compared to those of the original field populations (FST = 0.25 in Montero-Pau et al.64; FST = 0.18 in Franch-Gras et al.65). All but one population maintained their genetic diversity throughout the experiment. Nevertheless, the negligible loss of genetic diversity (H1) and the minor variation in FST values among populations suggest that genetic drift was of minor importance in comparison to selection66,67,68.

A strong signal of adaptation to laboratory conditions during the selection experiment was found. This was derived from the number of candidate SNPs under selection with parallel allelic frequency changes identified in all six laboratory populations regardless of the selective regime. These results were not unexpected65,66 given that (1) the laboratory populations were derived directly from the field and (2) the laboratory conditions other than the selective regime were identical and consistent across the selective regimes in the evolution experiment (e.g., temperature, salinity, food type and availability, light, length of dry phase, etc.). This contrasts with the different and highly variable conditions experienced by the field populations66 from which the origin population was derived64. The selective regimes mimicked the extreme patterns of selection imposed by the environmental unpredictability in the hydroperiod length to which B. plicatilis natural populations are exposed66. We focused on the unpredictability of the hydroperiod length, as it is expected to exert a strong selection pressure on ecologically relevant traits of rotifer life history, such as the diapausing egg hatching fraction and the timing of sex47,48,67. However, unpredictability is likely generated by other environmental factors that might also be associated with hydroperiod length in natural populations (e.g., salinity, temperature, food, antagonists, etc.) and could be expected to create additional selective pressures that were not explored here.

Genomic signatures of rapid adaptation in laboratory populations through experimental evolution have been identified, supporting previous results showing rapid phenotypic divergence in rotifer populations, which evolved divergent life-history traits over a short time span46. Although other experimental evolution studies have shown adaptation to a range of selective pressures in rotifers44,68 and other well-known cyclical parthenogens, such as Daphnia69,70, the genomic basis has been little explored. Genomic signatures of adaptation to environmental anthropogenic stressors were found in D. magna using an experimental evolution approach62; however, the genomic basis of adaptation to environmental unpredictability is essentially unknown. To the best of our knowledge, only a recent study involving rotifer field populations identified genomic signatures of local adaptation to unpredictable environments45. Nonetheless, the genetic variants that could be key in the adaptation to unpredictable environments are not shared between the two studies. It appears unlikely that only three genes underlie the genetic architecture of the life-history trait response to environmental unpredictability, given that such traits are likely to be affected by large numbers of genes52. In this regard, it is worth noting that several limitations constrain our results: (1) genes across the genome with small effects may not be detected as significant outliers given the power of evolution experiments, (2) we use a sample of SNPs on a draft genome, (3) the experiment is not designed to detect elements of adaptation due to idiosyncratic changes in each population, (4) selective pressures associated with habitat unpredictability in the field might differ from those in our experimental evolution
approach\(^7\), and (5) we recognize that the adaptation was to a particular pattern of unpredictable environment rather than to environmental unpredictability itself. To assess adaptation to environmental unpredictability (and its genomic basis), several replicated patterns of unpredictability would have to be included (of course implying greater complexity in the logistics of the experiment). These limitations could explain the discrepancies found between the results reported by Franch-Gras et al.\(^45\) and those of the present study.

The recent availability of a draft genome for \(B.\) plicatilis\(^45\) allowed us to call SNPs more confidently and to identify putative genes under selection. Although tentative functionality has been assigned to most of the genes containing those SNPs (53.3%), the lack of functional annotation for many of the genes of \(B.\) plicatilis precludes the understanding of the mechanisms under selection in this rotifer species.

In conclusion, we have shown genomic evidence of rapid adaptation in experimental populations of the rotifer \(B.\) plicatilis, building on previous results for ecologically relevant traits. Candidate SNPs under selection from a particular unpredictable fluctuation pattern were identified, some of them associated with key life-history traits in the life cycle of this rotifer species. Given that these responses allow monogonont rotifers to adapt to unpredictable environments, our results can serve as a basis for future studies focused on adaptation. In addition, the rotifer populations showed strong genomic signals of adaptation to laboratory conditions. Furthermore, this study shows the potential of using monogonont rotifer populations to evaluate signatures of adaptation in short-term experiments. Nonetheless, (1) a better understanding of the \(B.\) plicatilis genome and transcriptome and (2) experimental validation of the predicted genes are still necessary to elucidate the genomic basis of rotifer adaptation to unpredictable environments.

Material and Methods

Experimental laboratory populations and study clones. The populations analyzed were obtained from the last cycle of the evolution experiment described in Tarazona et al.\(^46\), in which the effects of environmental unpredictability on diapause-related traits in rotifers were tested. Briefly, in that study, six genetically identical multiclonal laboratory populations of the rotifer \(B.\) plicatilis were founded by placing together asexual females from each of 30 clonal lines from nine Spanish Mediterranean salt ponds and lakes (a total of 270 clones\(^43\)). These populations were subjected to two contrasting selective regimes (predictable vs unpredictable) during seven growing cycles of selection mimicking planktonic growing seasons. These growing cycles were interrupted by periods of habitat unsuitability—dry periods—(see Fig. 4). Three laboratory populations (functioning as replicates) were randomly assigned to the predictable regime, characterized by a regular hydroperiod length, and the other three were assigned to the unpredictable regime, characterized by a random hydroperiod length. The end of each growing cycle in both selective regimes was simulated by filtering the cultures and allowing the filtrate, containing diapausing eggs, to dry. Only the diapausing eggs were able to survive these drying events and restart the following growing cycle through the hatchlings after a period of diapause. Note that the unpredictable fluctuation pattern (i.e., the particular sequence of different growing cycle lengths) was the same for the three replicate populations, so we tested the effect of this particular pattern of fluctuation and not any unpredictable fluctuation in the growing cycle length. Notably, a few growing cycles in the unpredictable regime were so short that few or no new diapausing eggs were produced, so populations relied on hatchlings from the accumulated egg bank. These cycles impose a strong selective pressure in our experimental design. Precisely, the fact that within the randomly established sequence, there was an extremely short cycle in which the production of a new cohort of diapausing eggs is not possible allows the assessment of whether (1) the persistence of rotifer populations depends on the existence of the egg bank and (2) the rotifers experience a truly adverse cycle that they cannot anticipate. By including
several replicated experimental populations within each selective regime in the experimental evolution design, it was possible to evaluate their independent evolutionary trajectories. The experiment lasted 392 days—including growing cycles plus diapause periods (for further details, see Tarazona et al.46).

After the seventh cycle of selection, a total of 30 clones from each of the six laboratory populations were used to found clonal lines (180 clones in total) to carry out the DNA extraction. It is worth noting that 10 of these clones were also used to perform the life-history trait assays described in Tarazona et al.46.

DNA extraction. To obtain sufficient biomass for DNA extractions, each individual experimental clone was grown under standard culture conditions (12 g L$^{-1}$ saline water, maintained at 20°C, and fed the microalga *Tetraselmis suecica* in 1.5 L plastic bottles for 15 days, starting from a low-density stock culture. On day 15, when an abundance of more than 15,000 individuals was reached in each bottle, the cultures were filtered out through a 30-μm Nytal mesh sieve. The retained rotifers were released and kept in saline water (12 g L$^{-1}$) for 24 hours to purge their digestive tract and minimize contamination with microalgal DNA. Thereafter, the rotifers were retained on a 30-μm Nytal mesh sieve, which was frozen in liquid nitrogen. DNA extraction was performed on the frozen rotifer biomass using a JETFLEX Genomic DNA purification kit (GENOMED, Löhne, Germany) following the manufacturer’s instructions. DNA quality was assessed on a 1% agarose gel, and the quantification of DNA was performed with a Qubit 2.0 fluorometer (Life Technologies, Thermo Fisher Scientific Inc.). A total of 169 clones were obtained with a sufficient DNA quality and concentration (>20 ng/μl of DNA) to apply the genotyping-by-sequencing protocol.

Genotyping by sequencing (GBS). GBS libraries were prepared and sequenced at the Institute for Genomic Diversity (IGD, Ithaca, NY, USA) following the protocol described by Elshire et al.47. In brief, (1) DNA samples were digested with the restriction enzyme *ApeKI*(GC[A-T]GC), (2) GBS libraries were constructed with unique barcodes for each clone, and (3) after pooling the samples, sequencing was carried out using an Illumina HiSeq. 2000/2500 (100 bp, single-end).

SNP calling and filtering. Raw sequence data quality was analyzed using FastQC version 0.11.5 (www.bioinformatics.babraham.ac.uk/projects/fastqc/). Additionally, the published GBS data from the 270 clones used as founders of the experimental laboratory populations (hereafter origin population) were downloaded and employed for comparison (accession number REGN00000000)45. SNPs were called from the raw DNA reads using the SNP calling v2.pipeline as implemented in TASSEL5.4. All reads were trimmed to 64 bp, and identical reads were collapsed into tags. Thereafter, these tags were aligned against a draft assembly of the *B. plicatilis* genome of 108 Mb (accession number REGN00000000)45 using the Burrows-Wheeler alignment tool (BW A)75, and the B. *were collapsed into tags. Thereafter, these tags were aligned against a draft assembly of the *B. plicatilis* genome of 108 Mb (accession number REGN00000000)45 using the Burrows-Wheeler alignment tool (BW A)75, and the SNPs were called from the aligned tags. The default parameters intended to apply a more conservative approach: the minimum length of an aligned pair (-aLen 30 instead of default 0) and the minimum locus coverage (-mlnLcov 0.8 instead of default 0.1).

The set of SNPs was filtered by quality using scripts modified from Franch-Gras et al.45 (see Supplementary Dataset 3 in Franch-Gras et al.45) and VCFtools76 to construct the final “individual (clone) x genotype” matrix. The following parameters were set: (1) a minimum coverage of six reads to call a genotype, (2) a minimum of 50% of the individuals of each population genotyped, (3) a minimum allele frequency (maf) higher than 1%, (4) only two alleles present (–min-alleles,–max-alleles), (5) average read depth <150 (–max-meanDP), and (6) heterozygotes in each SNP <60%.

Data analysis. Genetic structure. To assess the genome-wide genetic variation and differentiation, a principal component analysis (PCA) was performed on individual clone genotypes of all populations using the “ade-genet” package49 in R50 v. 3.2.2. To evaluate between-population differentiation and the effects of drift, the fixation index (F$_ST$) for each pairwise comparison was estimated. Additionally, the F$_ST$ value between selective regimes was calculated by grouping the three replicates (i.e., the three populations) under each selective regime. Other basic statistics, such as the expected heterozygosity (H$_e$), observed heterozygosity (H$_o$), and inbreeding coefficient (F$_IS$), for the origin and six experimental laboratory populations were estimated. F$_ST$ values, H$_e$, H$_o$, and F$_IS$ were estimated using the R package “hierfstat”75. The function pairwise.fst within this package computes Nei’s pairwise F$_ST$ between pairs of populations. Hardy–Weinberg equilibrium (HWE) was tested using VCFtools for each population. File format conversions were performed using PGDSpider81 and Plink82 v.1.9.

Candidate SNPs under selection. Putative selected loci were identified in BayeScan83 v.2.1. This genome-scan method identifies markers that show stronger divergence patterns between groups than would be expected under neutral genetic processes. Moreover, it estimates the posterior probability that a given SNP is affected by selection. This F$_ST$-based method is a differentiation method that is robust to confounding demographic processes84,85 and has a low number of false positives86 relative to other methods. We carried out two BayeScan analyses, BS1 and BS2, using prior odds (PO) of 100 and 10 and a false discovery rate (FDR) of 0.05. PO refers to the ratio of posterior probabilities, which indicates the likelihood of the selection model in comparison to that of the neutral model87,88. Therefore, here, the selection model must be 10 or 100 times more likely than the neutral model for a locus to be considered as a candidate of being under selection. In BS1, the following groupings of populations were established: (1) origin populations, (2) the three replicate populations that evolved under the predictable regime (hereafter predictable populations), and (3) the three replicate populations that evolved under the unpredictable regime (hereafter unpredictable populations). In BS2, to assess the assignment of candidate SNPs to each selective regime (predictable vs unpredictable), we used two groupings: (1) predictable populations and (2) unpredictable populations. This design allowed us to observe parallel adaptive responses in replicate populations.
to obtain relatively robust results, but it did not allow us to identify idiosyncratic—population-specific—changes in the populations.

**Genome-wide association analysis (GWAS).** Genotype-phenotype association analysis was performed for the two life-history traits—timing of sex and hatching fraction of diapausing eggs—obtained for each clone in our previous experimental evolution study. We included clones with both phenotypic and genotypic data available (52 and 76 clones for timing of sex, respectively; Supplementary Dataset S2). In this dataset, the timing of sex is expressed as females mL−1, and the hatching fraction of diapausing eggs is expressed as the percentage of hatching eggs. Phenotype-genotype association analysis was performed by running linear models using Plink version 1.9 (Supplementary Method S1). The SNP dataset was filtered for a minimum allele frequency lower than 0.01. We implemented a stratified analysis controlling by population assignment and adjusting p-values with the Bonferroni correction (<0.05). Linkage disequilibrium (LD) was also assessed for candidate SNPs associated with both phenotypic traits using Plink.

**Identification of candidate genes under selection.** Gene functions were retrieved from the current annotation of the B. plicatilis genome. Genes putatively associated with the candidate SNPs identified with BayeScan and those identified with GWAS were identified in the flanking regions of 0, 2.5 and 5 kbp upstream and downstream of the focal SNPs using BEDTools. Additionally, a gene ontology (GO) enrichment analysis (Fisher's exact test, two-tailed, false discovery rate <0.05) was performed for those genes in which candidate SNPs under selection were found using Blast2GO.

**Data availability**

The sequences used in this study were deposited in GenBank (BIOPROJECT ID: PRJNA503132). GBS raw sequences were deposited in the ENA (Accession Number SRR8144248).

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**Author contributions**

Project was conceived by M.J.C., E.G.-R. and A.G. DNA extractions were carried out by E.T. The bioinformatic data analysis was performed by E.T. with the support of C.H., L.F.-G. and A.G. The first draft of the manuscript was written by A.G. and E.T. All authors discussed the results, contributed to subsequent manuscript drafts and approved the final manuscript.

**Competing interests**

The authors declare no competing interests.

**Additional information**

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