Linking Allele-Specific Expression And Natural Selection In Wild Populations

Romuald Laso-Jadart[1,6*], Kevin Sugier[1], Emmanuelle Petit[2], Karine Labadie[2], Pierre Peterlongo[3], Christophe Ambroise[4], Patrick Wincker[1,6], Jean-Louis Jamet[5], Mohammed-Amin Madoui[1,6*]

[1]Génomique Métabolique, Genoscope, Institut François Jacob, CEA, CNRS, Univ Evry, Université Paris-Saclay, Evry, France.

[2]CEA, Genoscope, Institut de Biologie François Jacob, Université Paris-Saclay, Evry, 91057, France.

[3]Univ Rennes, CNRS, Inria, IRISA - UMR 6074, F-35000 Rennes.

[4]LaMME, CNRS, Univ Evry, Université Paris-Saclay, Evry, France

[5]Université de Toulon, Aix-Marseille Université, CNRS/INSU/IRD, Mediterranean Institute of Oceanology MIO UMR 110, CS 60584, 83041 Toulon cedex 9, France.

[6]Research Federation for the study of Global Ocean Systems Ecology and Evolution, FR2022/Tara Oceans GO-SEE, 3 rue Michel-Ange, 75016 Paris, France

[*]Corresponding authors. Emails: rlasojad@genoscope.cns.fr & amadoui@genoscope.cns.fr

Abstract

Allele-specific expression (ASE) is now a widely studied mechanism at cell, tissue and organism levels. However, population-level ASE and its evolutive impacts have still never been investigated. Here, we hypothesized a potential link between ASE and natural selection on the cosmopolitan copepod *Oithona similis*. We combined metagenomic and metatranscriptomic data from seven wild populations of the marine copepod *O. similis* sampled during the *Tara* Oceans expedition. We detected 587 single nucleotide variants (SNVs) under ASE and found a significant amount of 152 SNVs under ASE in at least one population and under selection across all the populations. This constitutes a first evidence that selection and ASE target more common loci than expected by chance, raising new questions about the nature of the evolutive links between the two mechanisms.

Introduction

Allele-specific expression (ASE), or allelic imbalance, refers to difference of expression between two alleles of a locus in a heterozygous genotype due to genetic or epigenetic polymorphism. Through DNA methylation or histone modifications, epigenetics could repress a disadvantageous or a specific parental allele, leading in some cases to monoallelic expression, as demonstrated in a variety of organisms including mouse, maize or bumblebee [1–4]. On the other hand, ASE may have a genetic origin through, for example, mutations in transcription factor binding sites [5,6], or post-transcriptional mechanisms like non-sense mediated decay [7–9]. Recently, several studies led to a better understanding of ASE thanks to the development of advanced tools allowing their detection at the individual, tissue and cell levels[7,9–15]. ASE has been investigated in the context of *cis*- and *trans*-regulation of gene expression[16], expression evolution [17] and association between gene expression and human diseases [18]. First approaches in natural populations of primates and flycatchers have been undertaken with individual-level data [19–21]. Moreover, studies began to question the relative contribution of genetics and environment on gene expression using ASE in human [22–25]and fruit flies[26].

However, population-level ASE in several wild populations of one species and its potential evolutive origins and consequences remain largely uninvestigated. The need for numerous individual RNA-seq and whole-genome genotyping data constitutes the main obstacle for population-scale analyses. Today, the advances of next-generation sequencing technologies allow integrating large metagenomic and metatranscriptomic data from environmental samples, and new approaches can now be considered using whole population information.

In natural populations, we expect most loci to be under neutral evolution and balanced expression (Fig. 1a)[27,28]. When selection occurs on a specific locus, the selected allele tends to homozygosity

creating a specific population-level expression pattern of the selected allele (Fig.1b).In the absence of selection, if the same allele is favored by ASE in most of the individuals, the observed population-level expression pattern (Fig. 1c) will be similar to the one observed in the case of selection (Fig.1b).Considering that both mechanisms impact fitness, we hypothesized that ASE and natural selection could preferentially target the same loci, in different populations, showing a possible link between the two mechanisms.

In this study, we focus on the widespread epipelagic, temperate and cold water small-sized copepod, *Oithona similis* (Cyclopoida, Claus 1866), notably known to be highly abundant in Arctic[30–33]. Copepods, and particularly *Oithona*, are small crustaceans forming the most abundant metazoan on Earth, reflecting strong adaptive capacities to environmental fluctuations[34–36].They play a key ecological role in biogeochemical cycles and in the marine trophic food chain[37]; therefore copepods constitute an ideal model to study wild population evolution[38–41]

The first goal of our study was to identify loci under selection before demonstrating that population-level ASE can be detected with metagenomic and metatranscriptomic data collected by the *Tara* Oceans expedition[42] during its Arctic phase. Then we provided evidence of a quantitative link between ASE and natural selection.

Material and Methods

Material sampling, mRNA extraction and transcriptome sequencing

Oithona similis specimens were sampled at the North of the Large Bay of Toulon, France (Lat 43°06’ 02.3” N and Long 05°56’ 53.4”E). Sampling took place in November 2016. The samples were collected from the upper water layers (0-10m) using zooplankton nets with a mesh of 90µm

and 200µm (0.5 m diameter and 2.5 m length). Samples were preserved in 70% ethanol and stored at -4°C. From the Large Bay of Toulon samples, *O. similis* individuals were isolated under the stereomicroscope. We selected two different development stages: four copepodites (juveniles) and four adult males. Each individual was transferred separately and crushed, with a tissue grinder (Axygen) into a 1.5 mL tube (Eppendorf). Total mRNAs were extracted using the 'RNA isolation' protocol from NucleoSpin RNA XS kit (Macherey-Nagel) and quantified on a Qubit 2.0 with a RNA HS Assay kit (Invitrogen) and on a Bioanalyzer 2100 with a RNA 6000 Pico Assay kit (Agilent). cDNA were constructed using the SMARTer-Seq v4 Ultra low Input RNA kit (ClonTech). The libraries were constructed using the NEBNext Ultra II kit, and were sequenced with an Illumina HiSeq2500 (Supplementary Fig. 1).

Transcriptomes assembly and annotation

Each read set was assembled with Trinity v2.5.1[43] using default parameters and transcripts were clustered using cd-hit v4.6.1[44] (Supplementary Table 1).To ensure the classification of the sampled individuals, each ribosomal read set were detected with SortMeRNA [45] and mapped with bwa v0.7.15using default parameters [46] to 82 ribosomal 28S sequences of *Oithona* species used in Cornils et al., 2017 (Supplementary Fig. 2). The transcriptome assemblies were annotated with Transdecoder v5.1.0[43] to predict the open reading frames (ORFs) and protein sequences (Supplementary Table 1). In parallel, homology searches were also included as ORF retention criteria; the peptide sequences of the longest ORFs were aligned on *Oithona nana* proteome [40] using DIAMOND v0.9.22[48]. Protein domain annotation was performed on the final ORF predictions with Interproscan v5.17.56.0[49] and a threshold of e-value $<10^{-5}$ was applied for Pfam annotations. Finally, homology searches of the predicted proteins were done against the nr NCBI

database, restricted to Arthropoda (taxid: 6656), with DIAMOND v0.9.22 (Supplementary Fig. 1).

Variant calling using *Tara* Oceans metagenomic and metatranscriptomic data

We used metagenomic and metatranscriptomic reads generated from samples of the size fraction 20–180 µm collected in seven *Tara* Oceans stations TARA_155, 158, 178, 206, 208, 209 and 210 (Supplementary Table 2), according to protocols described in Alberti et al. 2017 [50].

The reference-free variant caller *DiscoSNP++* [51,52] was used to extract SNVs simultaneously from raw metagenomic and metatranscriptomic reads, and ran using parameters –b 1. Only SNVs corresponding to biallelic loci with a minimum of 4x of depth of coverage in all stations were initially selected. Then, SNVs were clustered based on their loci co-abundance across samples using density-based clustering algorithm implemented in the R package dbscan [53,54] and ran with parameters epsilon = 10 and minPts = 10. This generated three SNVs clusters, the largest of which contained 102,258 SNVs. To ensure the presence of *O. similis* SNVs without other species, we observed the fitting of the depth of coverage to the expected negative binomial distribution in each population (Supplementary Fig. 3). For each variant in each population, the B-allele frequency (BAF) and the population-level B-allele relative expression (BARE) were computed; $BAF = \frac{G_B}{G_B + G_A}$ and $BARE = \frac{T_B}{T_B + T_A}$, with G_A and G_B the metagenomic read counts of the reference and alternative alleles respectively, T_A and T_B the metatranscriptomic read counts of the reference and alternative alleles respectively.

Variant filtering and annotation

SNVs were filtered based on their metagenomic coverage. Those with a metagenomic coverage lying outside a threshold of median ± 2 σ in at least one population, with a minimum and

maximum of 5x and 150x coverage were discarded. To keep out rare alleles and potential calling errors, only variants characterized by a BAF comprised between 0.9 and 0.1, and a BARE between 0.95 and 0.05 in at least one population were chosen for the final dataset resulting in 25,768 SNVs (Supplementary Fig. 4).

The variant annotation was conducted in two steps. First, the variant sequences were relocated on the previously annotated *O.similis* transcripts using the "VCF_creator.sh" program of *DiscoSNP++*. Secondly, a variant annotation was carried with SNPeff [55] to identify the location of variants within transcripts (i.e., exon or UTR) and to estimate their effect on the proteins (missense, synonymous or nonsense).The excess of candidate variant annotations was tested in the following classes: missenses, synonymous, 5' and 3'UTR. A significant excess was considered for a hypergeometric test p-value < 0.05.

Genomic differentiation and detection of selection

The differentiation among the seven populations was investigated through the computation of the F_{ST} metric or Wright's fixation index [56,57].For each locus, global F_{ST} including the seven populations and pairwise-F_{ST} between each pair of population was computed, using the corresponding BAF matrix. For the global F_{ST} computation, a Hartigans' dip test for unimodality was performed [58]. We retained the median pairwise-F_{ST} as a measure of the genomic differentiation between each population. The *pcadapt* R package v4.0.2 [59] was used to detect selection among populations from the B-allele frequency matrix. The computation was run on "*Pool-seq*" mode, with a minimum allele frequency of 0.05 across the populations, and variants with a corrected Benjamini and Hochberg [60] p-value < 0.05 were considered under selection.

Population-level ASE detection using metagenomic and metatranscriptomic data

In each population, we first selected variants for BAF ≠ {0,1}. Then, we computed $D = BAF - BARE$, as the deviation between the BAF and the BARE. In the absence of ASE, D is close to 0, as most of the biallelic loci are expected to have a balanced expression[7,28,61] we expect the D distribution to follow a Gaussian distribution centered on 0. We estimated the Gaussian distribution parameters and tested the probability of a variant to belong to this distribution ("*D*-test" or "deviation test"). Given the large number of tests, we applied the Benjamini and Hochberg approach to control the False Discovery Rate (FDR).

We also computed a "low expression bias" test by comparing the read counts T_A and T_B to the observed metagenomic proportion 1-BAF and BAF respectively with a chi-square test and applied the Benjamini and Hochberg correction for multiple testing. These two tests were applied to BAFs, BAREs and read counts of the seven populations separately and the seven sets of candidate loci targeted by ASE (deviation test q-value < 0.1 and low expression bias test q-value< 0.1) were crossed to identify loci under ASE in different populations, or shared ASEs.

To identify alleles targeted by both ASE and selection, the set of variants under ASE in each population was crossed with the set of loci detected under selection. The size of the intersection was tested by a hypergeometric test, Hypergeometric(q,m,n,k), with q being number of alleles under ASE in the population and under selection (size of intersection), m being the total number of alleles under selection, n being the total number of variants under neutral evolution, and k being the total number of alleles under ASE in the tested population. We considered that, in a given population, the number of alleles under both ASE and selection was significantly higher than expected by chance for p-value < 0.05.

Gene enrichment analysis

To identify specific biological function or processes associated to the variants, a domain-based analysis was conducted. The Pfam annotation of the transcripts carrying variants targeted by ASE and selection was used as entry for dcGO Enrichment [62]**.** A maximum of the best 300 GO-terms were chosen based on their z-score and FDR p-value ($<10^{-3}$) in each ontology category. To reduce redundancy, these selected GO-terms were processed using REVIGO[63], with a similarity parameter of 0.5 against the whole Uniprot catalogue under the SimRel algorithm. To complete the domain-based analysis, the functional annotations obtained from the homology searches against the nr were manually curated.

Results

Oithona similis genomic differentiation and selection in Arctic Seas

From metagenomic and metatranscriptomic raw data of seven sampling stations (Fig. 2a), we identified 25,768 expressed variants. Among them, 97% were relocated on *O. similis* transcriptomes.

The global distribution of F_{ST} of the seven populations was unimodal (Hartigans' dip test, D=0.0012, p-value=0.99) with a median-F_{ST} at 0.1, confirmed by the pairwise-F_{ST} distributions (Supplementary Fig. 5).The seven populations were globally characterized by a weak to moderate differentiation, with a maximum median pairwise-F_{ST} of 0.12 between populations from TARA_210 and 155/178 (Fig. 2c,d). Populations from stations TARA_158 (Norway Current), 206 and 208 (Baffin Bay) were genetically closely-related, with the lowest median pairwise F_{ST} (0.02), despite TARA_158 did not co-geolocalize with the two other stations. The four other populations (TARA_155, 178, 209, and 210) were equally distant from each other (0.1-0.12).

Finally, TARA_158, 206 and 208 on one side, and TARA_155, 178, 210 and 209 on the other side showed the same pattern of differentiation (0.05-0.07).

The PCA decomposed the genomic variability in six components; the first two components discriminated TARA_155 and 178 from the others (32% and 28.1% variance explained respectively, Fig. 2b), and the third component differentiated TARA_210 and 209 (19.5%). The fourth principal component separated TARA_209 and 210 from 158/206/208 (11.3 %), with the last two concerning TARA_158/206/208 (Supplementary Fig. 5).Globally, these results dovetailed with the F_{ST} analysis, with details discussed later. Finally, we detected 674 variants under selection, representing 2.6% of the dataset (corrected p-value < 0.05).

Loci targeted by population-level ASE and selection in Arctic populations

The number of SNVs tested for ASE varied between 13,454 and 22,578 for TARA_210 and 206 respectively. As expected, the *D* deviation, representing the deviation between B-allele frequency and B-allele relative expression, followed a Gaussian distribution in each population (Fig. 3a and Supplementary Fig. 6). Variants under ASE (i.e. having a *D* significantly higher or lower than expected) were found in every population, ranging from 26 to 162 variants for TARA_178 and 206 respectively (Table 1). Overall, we found 587 variants under ASE, including 535 population-specific ASEs, and 52 ASEs shared by several populations (Fig.3b). Remarkably, 30 ASEs out of the 52 were present in the populations from TARA_158, 206 and 208 that correspond to the genetically closest populations. The seven sets of variants under ASE were crossed with the set of variants under selection, as illustrated for TARA_209 (Fig. 3c). The size of the intersection ranged from 5 to 42 variants (TARA_155 and 210/206) and was significantly higher than expected by chance for all the populations (hypergeometric test p-value < 0.05). It represented a total of 152 unique variants under selection and ASE in at least one population (Table 1,

Supplementary Table 3), corresponding to 23% and 26% of variants under ASE and under selection respectively.

Functional analysis of genes targeted by ASE and selection

Among the 152 loci targeted by ASE and selection, 145 were relocated on *O. similis* transcripts (Supplementary Table 4). Amid these transcripts, 137 (90%) had a predicted ORF, 97 (64%) were linked to at least one Pfam domain and 90 (59%) to a functional annotation. Fifteen SNVs were missense variations, 59 synonymous, 31 and 29 were located in 5' and 3' UTR, without any significant excess (Supplementary Table 4 and 5). Based on homology searches (Supplementary Table 4), eight genes were linked to nervous system (Table2). Among them, two genes were involved in glutamate metabolism (omega-amidase NIT2 and5-oxoprolinase), three were predicted to be glycine, γ-amino-butyric acid (GABA) and histamine neuroreceptors. Finally, four were also implicated in arthropods photoreceptors. The domain-based analysis confirmed these results, with an enrichment in GO-terms biological process also linked to nervous system (Supplementary Fig. 7).

Discussion

***O. similis* populations are weakly structured within the Arctic Seas**

Global populations of *O. similis* are known to be composed of cryptic lineages[47]. Thus the assessment that the seven populations used in our study belong to the same *O. similis* cryptic lineage was a prerequisite for further analyses. The high proportion of variants mapped on the Mediterranean transcriptomes (97%) showed that the variant clustering method was efficient to regroup loci of an *O.similis* cryptic species. Plus, the unimodal distribution of F_{ST} showed that these populations of *O. similis* belong to the same polar cryptic species.

Secondly, we see that the seven populations examined are well connected with low median pairwise-$F_{ST,}$ despite the large distances separating them. Weak genetic structure in the polar region was already highlighted for other major Arctic copepods like *Calanus glacialis*[64], and *Pseudocalanus* species[65]. The absence of structure was explained by ancient diminutions of effective population size due to past glaciations[65–67], or high dispersal and connectivity between the present-day populations due to marine currents[64].

Going into details, three different cases can be described. First, the differentiation of populations from TARA_155 and 178 compared to the others could be explained by isolation-by-distance. Secondly, the geographically close populations from TARA_210 and 209 present higher differentiation (median pairwise-F_{ST} of 0.11). This could be explained by the West Greenland current acting as a physical barrier between the populations, which could lead to reduced gene flow[68]. At last, the strong link between TARA_158 from Northern Atlantic current and TARA_206/208 from the Baffin Bay is the most intriguing. Despite the large distances that separate the first one from the others, these three populations are well connected.

Metagenomic data enable to draw the silhouette of the population genetics but lacks resolution when dealing with intra-population structure. However, our findings are concordant with previous studies underpinning the large-scale dispersal, interconnectivity of marine zooplankton populations in other oceans, at diverse degrees[38,69–71].

Toward the link between ASE and natural selection

Usually, at the individual level, the ASE analyses are achieved by measuring the difference in RNA-seq read counts of a heterozygous site. But at the population level, obtaining a large number of individuals remains a technical barrier especially for uncultured animals, or when the

amount of DNA retrieved from a single individual is not sufficient for high-throughput sequencing. Here, the detection of ASE at population level was possible by comparing the observed frequencies of the alleles based on metagenomic and metatranscriptomic data, which by passes the obstacles previously described.

In our study, the amount of detected ASE in each population was always lower than 1% of tested heterozygous variants, which altogether correspond to 2% of the total set of variants. In humans [72],baboons [21] and flycatchers [19], 17%, 23% of genes and 7.5% of transcripts were affected by ASE respectively. The difference with our results can be explained by one main reason. The detection of population-level ASE identifies only the ASE present in a large majority of individuals, which can be considered as “core ASEs”.

These core ASEs constitute the majority of detected ASEs and are population-specific, meaning the main drivers of this expression pattern are local conditions like different environmental pressures or population dynamics including, for example, the proportion of each developmental stage and sex, known to vary between populations and across seasons [30,73]. Another result is the presence of a small amount of variants affected by ASE in different populations. Most of these variants are under ASE in at least two of the three closest populations from TARA_158, 206 and 208. First, the genetic closeness and large geographic distances between these three populations suggest that their shared ASEs are under an environmental independent genetic control. Secondly, the number of variants tested for ASE is higher in these three populations than the others, leading to a greater proportion of ASEs detected which also elevates the chances for a variant to be declared under ASE in several populations.

A significant amount of SNVs (152) were subject to selection among the seven populations and to ASE in at least one population. We confirmed our first hypothesis (Fig. 1), as exampled with the variant 841109 (Fig. 3d), characterized by an ASE in favor of the B-allele in TARA_209 and fixation of this allele in TARA_210. Three main features of ASE can be under selection. First, the observed variant can be in linkage with another variation in upstream *cis*-regulatory elements like transcription factors fixation sites, or epialleles[7]. Secondly, the annotation of candidate variants with SNPeff revealed a majority of variants located in 5' and 3'UTRs, which are variations known to both affect transcription efficiency through mRNA secondary structures, stability and location[74–76]. For variants located in exons, a majority were identified as synonymous mutations, growingly described as potential target of selection by codon usage bias, codon context, mRNA secondary structure or transcription and translation dynamics [77,78]. Finally, fifteen missense mutations were spotted, but with moderate predicted impact on protein amino acid composition. However, we did not find premature nonsense mutation, even if variants under ASE has been described to trigger or escape potential nonsense-mediated decay [28,61,79], but the possibility that the causal variation is located in introns cannot be ruled out.

The process of adaptation through gene expression was suspected in human populations and investigated thanks to the large and accessible amount of data. In a first study, a link has been established between gene expression and selection, affecting particular genes and phenotypes, looking at *cis*-acting SNPs[80]. In a second study, the team was able to detect ASE in different populations and to quantify genetic differentiation and selection[61]. They found particularly one gene that shows strong differentiation between European and African populations and under ASE in Europeans and not in Africans. However, they did not quantify this phenomenon. Both emphasized the impact of selection on gene expression. In the same way, another approach

showed that ASE or expression variations with high effect size were rare in the populations, based on intra-population analyses in *Capsella grandiflora* and human [28,81]. This situation is presumably encountered in our analysis, as exampled with the B-allele of variant 20760212, under ASE and with a low genomic frequency in TARA_210, but fixed in the others (Fig. 3d). Our results complete previous analyses, as they quantify the link between ASE and selection in populations and reveal the evolutive potency of ASE, for the first time at the population-level. It remains to understand the nature of the association between ASE and selection. To address this question, we formulate the hypothesis that they impact chronologically the same loci, following constant or increasing selective pressure as well as environmental changes (Fig. 4).

Nervous system and visual perception are important targets of the natural selection and ASE in *O. similis*

This evolutive link between ASE and selection is supported by the biological functions associated to the targeted genes, which are involved notably in the copepods nervous system in two ways. The first result is the presence of genes implicated in glutamate metabolism and glycine and/or GABA receptors. Glutamate and GABA are respectively excitatory and inhibitory neurotransmitters in arthropods motor neurons [82]. Plus, glycine and GABA receptors have already been described as a target of selection in *O. nana* in Mediterranean Sea[40,41]. Secondly, the functional analysis revealed also the importance of the eye and visual perception in the *O. similis* evolution.

Copepod nervous system constitutes a key trait for its reproduction and survival, and based on our data, a prime target for evolution, allowing higher capacity of perceiving and fast reacting leading to more efficient predator escape, prey catching and mating. This can explain the great evolutive success of these animals [35,83,84].

Conclusion

Gene expression variation is thought to play a crucial role in evolutive and adaptive history of natural populations. Herein, we developed proper methods integrating metagenomic and metatranscriptomic data to detect ASE at the population-level for the first time. Then, we demonstrated the link between ASE and natural selection by providing a quantitative observation of this phenomenon and its impact on specific biological features of copepods. In the future, we will try to generalize these observations to other organisms. Then, we will understand the nature of the link between ASE and natural selection by questioning the chronology between the two mechanisms.

Acknowledgments

We thank the people and sponsors who participated in the *Tara* Oceans Expedition 2009–2013: Centre National de la Recherche Scientifique, European Molecular Biology Laboratory, Genoscope/Commissariat à l'Energie Atomique, the French Government "Investissements d'Avenir" programmes OCEANOMICS (ANR-11- BTBR-0008), FRANCE GENOMIQUE (ANR-10-INBS-09-08), Agnes b., the Veolia Environment Foundation, Region Bretagne, World Courier, Illumina, Cap L'Orient, the Electricite de France (EDF) Foundation EDF Diversiterre, Fondation pour la Recherche sur la Biodiversite, the Prince Albert II de Monaco Foundation, Etienne Bourgois and the *Tara* schooner and its captain and crew. *Tara* Oceans would not exist without continuous support from 23 institutes (oceans.tara-expeditions.org). This is contribution number XX from *Tara* Oceans.

Author's contributions

Individuals for transcriptome production were sampled by J-LJ and KS. KS extracted RNA, EP and KL prepared the libraries and sequencing, MAM assembled the reads and RLJ annotated transcriptomes. PP and CA gave expertise support on *DiscoSNP++* and statistical framework respectively. RLJ and MAM performed the analyses and wrote the manuscript. MAM designed and supervised the study. J-LJ and PW offered scientific support.

Competing interests

The authors declare no competing interests.

References

1. Szabo, P. E. & Mann, J. R. Allele-specific expression and total expression levels of imprinted genes during early mouse development: implications for imprinting mechanism. *Genes Dev.* **9,** 3097–3108 (1995).

2. Wei, X. & Wang, X. A computational workflow to identify allele-specific expression and epigenetic modification in maize. *Genomics, Proteomics Bioinforma.* **11,** 247–252 (2013).

3. Ginart, P. *et al.* Visualizing allele-specific expression in single cells reveals epigenetic mosaicism in an H19loss-of-imprinting mutant. *Genes Dev.* **30,** 567–578 (2016).

4. Lonsdale, Z. *et al.* Allele specific expression and methylation in the bumblebee, *Bombus terrestris*. *PeerJ* **5,** e3798 (2017).

5. Bailey, S. D., Virtanen, C., Haibe-Kains, B. & Lupien, M. ABC: A tool to identify SNVs causing allele-specific transcription factor binding from ChIP-Seq experiments. *Bioinformatics* **31,** 3057–3059 (2015).

6. Cavalli, M. *et al.* Allele-specific transcription factor binding to common and rare variants associated with disease and gene expression. *Hum. Genet.* **135,** 485–497 (2016).

7. Castel, S. E., Levy-Moonshine, A., Mohammadi, P., Banks, E. & Lappalainen, T. Tools and best practices for data processing in allelic expression analysis. *Genome Biol.* **16,** 195 (2015).

8. Rivas, M. A. *et al.* Impact of predicted protein-truncating genetic variants on the human

transcriptome. *Science (80-.).* **348,** 666–669 (2015).

9. Pirinen, M. *et al.* Assessing allele-specific expression across multiple tissues from RNA-seq read data. *Bioinformatics* **31,** 2497–2504 (2015).

10. Lu, R. *et al.* Analyzing allele specific RNA expression using mixture models. *BMC Genomics* **16,** 566 (2015).

11. Harvey, C. T. *et al.* QuASAR: Quantitative allele-specific analysis of reads. *Bioinformatics* **31,** 1235–1242 (2015).

12. Miao, Z., Alvarez, M., Pajukanta, P. & Ko, A. ASElux: An ultra-fast and accurate allelic reads counter. *Bioinformatics* **34,** 1313–1320 (2018).

13. Mayba, O. *et al.* MBASED: allele-specific expression detection in cancer tissues and cell lines. *Genome Biol.* **15,** 405 (2014).

14. Skelly, D. A., Johansson, M., Madeoy, J., Wakefield, J. & Akey, J. M. A powerful and flexible statistical framework for testing hypotheses of allele-specific gene expression from RNA-seq data. *Genome Res.* **21,** 1728–1737 (2011).

15. M. Dong, Y. J. Single-Cell Allele-Specific Gene Expression Analysis. *Comput. Methods Single-Cell Data Anal.* **1935,** (2019).

16. Ge, B. *et al.* Global patterns of cis variation in human cells revealed by high-density allelic expression analysis. *Nat. Genet.* **41,** 1216–1222 (2009).

17. Signor, S. A. & Nuzhdin, S. V. The Evolution of Gene Expression in cis and trans. *Trends Genet.* 1–13 (2018). doi:10.1016/j.tig.2018.03.007

18. McKean, D. M. *et al.* Loss of RNA expression and allele-specific expression associated with congenital heart disease. *Nat. Commun.* **7,** 1–9 (2016).

19. Wang, M., Uebbing, S. & Ellegren, H. Bayesian inference of allele-specific gene expression indicates abundant Cis-regulatory variation in natural flycatcher populations. *Genome Biol. Evol.* **9,** 1266–1279 (2017).

20. Howe, B., Umrigar, A. & Tsien, F. Chromosome Preparation From Cultured Cells. *J. Vis. Exp.* 3–7 (2014). doi:10.3791/50203

21. J. Tung, M. Y. Akinyi, S. Mutura, J. Altmann, G. A. W. and S. C. & Alberts. Allele-specific gene expression in a wild nonhuman primate population. *Mol. Ecol.* **2,** 147–185 (2015).

22. Buil, A. *et al.* Gene-gene and gene-environment interactions detected by transcriptome sequence analysis in twins. *Nat. Genet.* **47,** 88–91 (2014).

23. Cheung, V. G. *et al.* Monozygotic Twins Reveal Germline Contribution to Allelic Expression Differences. *Am. J. Hum. Genet.* **82,** 1357–1360 (2008).

24. Moyerbrailean, G. A. *et al.* High-throughput allele-specific expression across 250

environmental conditions. *Genome Res.* **26,** 1627–1638 (2016).

25. Knowles, D. A. *et al.* Allele-specific expression reveals interactions between genetic variation and environment. *Nat. Methods* **14,** 699–702 (2017).

26. Leon-Novelo, L., Gerken, A. R., Graze, R. M., McIntyre, L. M. & Marroni, F. Direct Testing for Allele-Specific Expression Differences Between Conditions. *G3 GENES, GENOMES, Genet.* **8,** g3.300139.2017 (2017).

27. Jensen, J. D. *et al.* The importance of the neutral theory in 1968 and 50 years on: a response to Kern & Hahn 2018. *Evolution (N. Y).* 1968–1971 (2018). doi:10.1111/evo.13650

28. Lappalainen, T. *et al.* Transcriptome and genome sequencing uncovers functional variation in humans. *Nature* **501,** 506–511 (2013).

29. Claus, C. Die Copepoden-Fauna von Nizza. Ein Beitrag zur Charakteristik der Formen und deren Abänderungen ‘im Sinna Darwin’s’. *Elwebt’sche Univ. Marbg. Leipzig* **1,** 1:34 (1866).

30. Dvoretsky, V. G. Seasonal mortality rates of Oithona similis (Cyclopoida) in a large Arctic fjord. *Polar Sci.* **6,** 263–269 (2012).

31. Castellani. Contribution to the Themed Section□: ‘ The Role of Zooplankton in Marine Biogeochemical Cycles□: From Fine Scale to Global Marine zooplankton and the Metabolic Theory of Ecology□: is it a predictive tool□? *J. Plankton Res.* **38,** 762–770 (2016).

32. Blachowiak-Samolyk, K., Kwasniewski, S., Hop, H. & Falk-Petersen, S. Magnitude of mesozooplankton variability: A case study from the Marginal Ice Zone of the Barents Sea in spring. *J. Plankton Res.* **30,** 311–323 (2008).

33. Zamora-Terol, S., Nielsen, T. G. & Saiz, E. Plankton community structure and role of Oithona similis on the western coast of Greenland during the winter-spring transition. *Mar. Ecol. Prog. Ser.* **483,** 85–102 (2013).

34. Humes, A. G. How Many Copepods? *Hydrobiologia* **293,** 1–7 (1994).

35. Kiørboe, T. What makes pelagic copepods so successful? *J. Plankton Res.* **33,** 677–685 (2011).

36. Gallienne, C. P. Is Oithona the most important copepod in the world’s oceans? *J. Plankton Res.* **23,** 1421–1432 (2001).

37. Wassmann, P. *et al.* Food webs and carbon flux in the Barents Sea. *Prog. Oceanogr.* **71,** 232–287 (2006).

38. Peijnenburg, K. T. C. A. & Goetze, E. High evolutionary potential of marine zooplankton. *Ecol. Evol.* **3,** 2765–2781 (2013).

39. Riginos, C., Crandall, E. D., Liggins, L., Bongaerts, P. & Treml, E. A. Navigating the

currents of seascape genomics: How spatial analyses can augment population genomic studies. *Curr. Zool.* **62,** 581–601 (2016).

40. Madoui, M. A. *et al.* New insights into global biogeography, population structure and natural selection from the genome of the epipelagic copepod Oithona. *Mol. Ecol.* **26,** 4467–4482 (2017).

41. Arif, M. *et al.* Discovering Millions of Plankton Genomic Markers from the Atlantic Ocean and the Mediterranean Sea. *Mol. Ecol. Resour.* 0–3 (2018). doi:10.1111/1755-0998.12985

42. Karsenti, E. *et al.* A Holistic Approach to Marine Eco-Systems Biology. *PLoS Biol.* **9,** e1001177 (2011).

43. Haas, B. J. *et al.* De novo transcript sequence reconstruction from RNA-seq using the Trinity platform for reference generation and analysis. *Nat. Protoc.* **8,** 1494–1512 (2013).

44. Fu, L., Niu, B., Zhu, Z., Wu, S. & Li, W. CD-HIT: Accelerated for clustering the next-generation sequencing data. *Bioinformatics* **28,** 3150–3152 (2012).

45. Kopylova, E., Noé, L. & Touzet, H. SortMeRNA: Fast and accurate filtering of ribosomal RNAs in metatranscriptomic data. *Bioinformatics* **28,** 3211–3217 (2012).

46. Li, H. & Durbin, R. Fast and accurate long-read alignment with Burrows-Wheeler transform. *Bioinformatics* **26,** 589–595 (2009).

47. Cornils, A., Wend-Heckmann, B. & Held, C. Global phylogeography of Oithona similis s.l. (Crustacea, Copepoda, Oithonidae) – A cosmopolitan plankton species or a complex of cryptic lineages? *Mol. Phylogenet. Evol.* **107,** 473–485 (2017).

48. Buchfink, B., Xie, C. & Huson, D. H. Fast and sensitive protein alignment using DIAMOND. *Nat. Methods* **12,** 59–60 (2014).

49. Jones, P. *et al.* InterProScan 5: genome-scale protein function classification. *Bioinformatics* **30,** 1236–1240 (2014).

50. Alberti, A. *et al.* Viral to metazoan marine plankton nucleotide sequences from the Tara Oceans expedition. *Sci. Data* **4,** 170093 (2017).

51. Uricaru, R. *et al.* Reference-free detection of isolated SNPs. *Nucleic Acids Res.* (2014). doi:10.1093/nar/gku1187

52. Peterlongo, P., Riou, C., Drezen, E. & Lemaitre, C. DiscoSnp++: de novo detection of small variants from raw unassembled read set(s). *bioRxiv* 209965 (2017). doi:10.1101/209965

53. Ester, M., Kriegel, H.-P., Sander, J. & Xu, X. *A Density-Based Algorithm for Discovering Clusters in Large Spatial Databases with Noise*. (1996).

54. Ram, A., Jalal, S., Jalal, A. S. & Kumar, M. A Density Based Algorithm for Discovering Density Varied Clusters in Large Spatial Databases. *Int. J. Comput. Appl.* **3,** 1–4 (2010).

55. Cingolani, P. and Platts, A. and Coon, M. and Nguyen, T. and Wang, L. and Land, S.J. and Lu, X. and Ruden, D. M. A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of Drosophila melanogaster strain w1118□; iso-2; iso-3. *Fly* **6,** 80–92 (2012).

56. B. S. Weir and C. Clark Cockerham. Estimating F-Statistics for the Analysis of Population Structure. *Evolution (N. Y).* **38,** 1358–1370 (1984).

57. Wright, S. the Genetical Structure of Populations. *Ann. Eugen.* **15,** 323–354 (1951).

58. Hartigan, J. A. & Hartigan, P. M. The dip test of unimodality. *Ann. Stat.* **13,** 70–84 (1985).

59. Luu, K., Bazin, E. & Blum, M. G. B. pcadapt: an R package to perform genome scans for selection based on principal component analysis. *Mol. Ecol. Resour.* **17,** 67–77 (2017).

60. Benjamini, Y. & Hochberg, Y. Controlling the False Discovery Rate□: A Practical and Powerful Approach to Multiple Testing. *J. R. Stat. Soc. Ser. B* **57,** 289–300 (1995).

61. Tian, L. *et al.* Genome-wide comparison of allele-specific gene expression between African and European populations. *Hum. Mol. Genet.* **27,** 1067–1077 (2018).

62. Fang, H. & Gough, J. DcGO: Database of domain-centric ontologies on functions, phenotypes, diseases and more. *Nucleic Acids Res.* **41,** (2013).

63. Supek, F., Bošnjak, M., Škunca, N. & Šmuc, T. Revigo summarizes and visualizes long lists of gene ontology terms. *PLoS One* **6,** (2011).

64. Weydmann, A., Coelho, N. C., Serrão, E. A., Burzyński, A. & Pearson, G. A. Pan-Arctic population of the keystone copepod Calanus glacialis. *Polar Biol.* **39,** 2311–2318 (2016).

65. Aarbakke, O. N. S., Bucklin, A., Halsband, C. & Norrbin, F. Comparative phylogeography and demographic history of five sibling species of Pseudocalanus (Copepoda: Calanoida) in the North Atlantic Ocean. *J. Exp. Mar. Bio. Ecol.* **461,** 479–488 (2014).

66. Edmands, S. Phylogeography of the intertidal copepod Tigriopus californicus reveals substantially reduced population differentiation at northern latitudes. *Mol. Ecol.* **10,** 1743–1750 (2001).

67. Bucklin, A. & Wiebe, P. H. Low mitochondrial diversity and small effective population sizes of the copepods Calanus finmarchicus and Nannocalanus minor: Possible impact of climatic variation during recent glaciation. *J. Hered.* **89,** 383–392 (1998).

68. Myers, P. G., Donnelly, C. & Ribergaard, M. H. Structure and variability of the West Greenland Current in Summer derived from 6 repeat standard sections. *Prog. Oceanogr.* (2008). doi:10.1016/j.pocean.2008.12.003

69. Blanco-Bercial, L. & Bucklin, A. New view of population genetics of zooplankton: RAD-seq analysis reveals population structure of the North Atlantic planktonic copepod Centropages typicus. *Mol. Ecol.* **25,** 1566–1580 (2016).

70. Höring, F., Cornils, A., Auel, H., Bode, M. & Held, C. Population genetic structure of

Calanoides natalis (Copepoda, Calanoida) in the eastern Atlantic Ocean and Benguela upwelling system. *J. Plankton Res.* **39,** 618–630 (2017).

71. Goetze, E. Global Population Genetic Structure and Biogeography of the Oceanic Copepods Eucalanus Hyalinus and E. Spinifer. *Evolution (N. Y).* **59,** 2378 (2005).

72. Zhang, S. *et al.* Genome-wide identification of allele-specific effects on gene expression for single and multiple individuals. *Gene* **533,** 366–373 (2014).

73. Lischka, S. & Hagen, W. Life histories of the copepods Pseudocalanus minutus, P. acuspes (Calanoida) and Oithona similis (Cyclopoida) in the Arctic Kongsfjorden (Svalbard). *Polar Biol.* **28,** 910–921 (2005).

74. Mignone, F., Gissi, C., Liuni, S., Pesole, G. & others. Untranslated regions of mRNAs. *Genome Biol* **3,** 4–1 (2002).

75. Dvir, S. *et al*. Deciphering the rules by which 5'-UTR sequences affect protein expression in yeast. *Proc. Natl. Acad. Sci.* **110,** E2792–E2801 (2013).

76. Matoulkova, E., Michalova, E., Vojtesek, B. & Hrstka, R. The role of the 3′ untranslated region in post-transcriptional regulation of protein expression in mammalian cells. *RNA Biol.* **9,** 563–576 (2012).

77. Shabalina, S. A., Spiridonov, N. A. & Kashina, A. Sounds of silence: Synonymous nucleotides as a key to biological regulation and complexity. *Nucleic Acids Res.* **41,** 2073–2094 (2013).

78. Ingvarsson, P. K. Natural Selection on Synonymous and Nonsynonymous Mutations Shapes Patterns of Polymorphism in Populus tremula. *Mol. Biol. Evol.* **27,** 650–660 (2010).

79. Rivas, M. A. *et al*. Effect of predicted protein-truncating genetic variants on the human transcriptome. *Science (80-.).* **348,** 666–669 (2015).

80. Fraser, H. B. Gene expression drives local adaptation in humans Gene expression drives local adaptation in humans. *Genome Res.* 1089–1096 (2013). doi:10.1101/gr.152710.112

81. Josephs, E. B., Lee, Y. W., Stinchcombe, J. R. & Wright, S. I. Association mapping reveals the role of purifying selection in the maintenance of genomic variation in gene expression. *Proc. Natl. Acad. Sci.* **112,** 15390–15395 (2015).

82. Smarandache-Wellmann, C. R. Arthropod neurons and nervous system. *Curr. Biol.* **26,** R960–R965 (2016).

83. Svensen, C. Remote prey detection in Oithona similis: hydromechanical versus chemical cues. *J. Plankton Res.* **22,** 1155–1166 (2000).

84. Kiørboe, T., Andersen, A., Langlois, V. J. & Jakobsen, H. H. Unsteady motion: Escape jumps in planktonic copepods, their kinematics and energetics. *J. R. Soc. Interface* **7,** 1591–1602 (2010).

85. Denno, M. E., Privman, E., Borman, R., Wolin, D. & Venton, B. J. Quantification of histamine and carcinine in Drosophila melanogaster tissues. *ACS Chem Neurosci* **7,** 407–414 (2016).

86. Monastirioti, M. Biogenic amine systems in the fruit fly Drosophila melanogaster. *Microsc. Res. Tech.* **45,** 106–121 (1999).

87. Stuart, A. E. From fruit flies to barnacles, histamine is the neurotransmitter of arthropod photoreceptors. *Neuron* **22,** 431–433 (1999).

88. Gurudev, N., Yuan, M. & Knust, E. chaoptin, prominin, eyes shut and crumbs form a genetic network controlling the apical compartment of Drosophila photoreceptor cells. *Biol. Open* **3,** 332–341 (2014).

89. Krantz, D. E. & Zipursky, S. L. Drosophila chaoptin, a member of the leucine-rich repeat family, is a photoreceptor cell-specific adhesion molecule. *EMBO J.* **9,** 1969–77 (1990).

90. Masai, I., Okazakit, A., Hosoyat, T. & Hottatt, Y. Drosophila retinal degeneration A gene encodes an eye-specific diacylglycerol kinase with cysteine-rich zinc-finger motifs and ankyrin repeats (signal transduction/phosphatidylinositol metabolism). *Neurobiology* **90,** 11157–11161 (1993).

91. Wang, T. & Montell, C. Phototransduction and retinal degeneration in Drosophila. *Pflugers Arch. Eur. J. Physiol.* **454,** 821–847 (2007).

92. Rawls, A. S. Strabismus requires Flamingo and Prickle function to regulate tissue polarity in the Drosophila eye. *Development* **130,** 1877–1887 (2003).

93. Leung, V. *et al.* The planar cell polarity protein Vangl2 is required for retinal axon guidance. *Dev. Neurobiol.* **76,** 150–165 (2016).

Tables

Table 1: Allele-specific expression detection and link with selection by population

Table 2: Functional annotations of variants targeted by ASE and selection implicated in nervous system

Supplementary Table 1: *Oithona similis* Mediterranean transcriptomes summary

Supplementary Table 2: *Tara* Oceans and *Oithona similis* Mediterranean transcriptomes samples accession numbers

Supplementary Table 3: Variants targeted by ASE and selection statistics

Supplementary Table 4: Variants targeted by ASE and selection functional annotations

Supplementary Table 5: Variant annotation by SNPeff

Figures

Figure 1: Population genomic and transcriptomic profiles of a biallelic locus in a case of **a,** Neutral evolution and balanced expression; **b,** Selection in favor of the B-allele; **c,** ASE in favor of the B-allele.

Figure 2: Genomic differentiation of *O. similis* populations from Arctic Seas. **a,** Geographic locations of the seven *Tara* Oceans sampling sites: Northern Atlantic (blue), Kara Sea (green), Baffin Bay (orange) and Labrador Sea (grey). **b,** Principal Component Analysis (PCA) computed by *pcadapt* based on allele frequencies. **c,** Pairwise-F_{ST} matrix. The median (mean) of each pairwise-F_{ST} distribution computed on allele frequencies is indicated. **d,** Graph representing the genomic differentiation of the seven populations of *O. similis*. The nodes represent the populations; their width reflects their centrality in the graph. The edges correspond to the genetic relatedness based on the median pairwise-F_{ST} between each pair of population; 0.02 (large solid line), 0.05 to 0.07 (thin solid line) and 0.11 to 0.12 (dashed line).

Figure 3: Population Allele-specific expression detection and link with natural selection. **a,** The deviation *D* distribution in TARA_209. The red line corresponds to the Gaussian distribution estimated from the data. **b,** Upset plot of the ASE detection in the seven populations. Each bar of the upper plot corresponds to the number of variants under ASE in the population(s) indicated by black dots in the lower plot. **c,** Crossing ASE and selection. The yellow circle represents the total set of variants. In green, the number of heterozygous variants tested for ASE in TARA_209. In blue and red, the amount of detected variants under ASE in TARA_209 and under selection among the populations respectively. In purple, the intersection comprising variants under ASE in TARA_209 and under selection, with its hypergeometric test p-value. **d,** Metagenomic and metatranscriptomic profiles of variants 841109 and 20760212. Each population is indicated on the x-axis, with the associated B-allele frequency (red) and B-allele relative expression (blue). The frequency is shown on the y-axis. The asterisks mean ASE was detected in the corresponding population.

Figure 4: From Allele-specific expression to natural selection. **a,** Evolution of allele frequency and allele relative expression over time. **b,** Evolution of selective pressure over time

Supplementary Fig 1: Method pipeline overview

Supplementary Fig 2: Validation of taxonomic assignation

Supplementary Fig 3: *Oithona similis* depth of coverage of biallelic loci in seven *Tara* Oceans samples

Supplementary Fig 4: Metagenomic coverage distribution of the seven *Tara* Oceans samples

Supplementary Fig 5: Genomic differentiation of Arctic Seas *Oithona similis* populations

Supplementary Fig 6: Allele-specific expression detection

Supplementary Fig 7: Functional analysis of *O. similis* transcripts targeted by ASE and selection

Table 1: Allele-specific expression detection and link with selection by population

Population	Genomic median depth of coverage	Number of tested variants	Number of variants under ASE	Number of variants under ASE and selection	Hypergeometric test p-value
TARA_155	25	18,812	91	6	9.89E-3*
TARA_158	35	21,476	131	29	5.06E-20*
TARA_178	24	18,145	26	9	5E-11*
TARA_206	55	22,578	162	42	4.82E-31*
TARA_208	48	21,469	133	14	2.2E-6*
TARA_209	12	13,956	62	24	1.05E-23*
TARA_210	14	13,454	69	42	8.89E-51*
Overall	-	25,768	587 (2.3%)	152 (0.59%)	-

Table 2: Functional annotations of variants targeted by ASE and selection implicated in nervous system

VarID	Ref	Alt	Homology search	Pfam	SnpEff Localization	SnpEff Impact	References
722267	A	G	histamine H1 receptor	PF00001	3 ' UTR	MODIFIER	[85–87]
9665345	T	G	chaoptin	PF13306 | PF13855	synonymous variant	LOW	[88,89]
15623788	G	A	eye-specific diacylglycerol kinase	PF13637	synonymous variant	LOW	[90,91]
23795359	A	T	vang-like protein 2-B	PF06638	synonymous variant	LOW	[92,93]
1276227	C	T	glycine receptor subunit alpha-2 / gamma-aminobutyric acid receptor subunit alpha-6	PF02932 | PF2931	3 ' UTR	MODIFIER	-
1404415	G	C	omega-amidase NIT2	PF00795	3 ' UTR	MODIFIER	-
11174785	A	G	5-oxoprolinase	PF02538 | PF05378 | PF01968	5 ' UTR	MODIFIER	-
11690229	A	T	glycine receptor subunit alpha-2	PF02931	synonymous variant	LOW	-

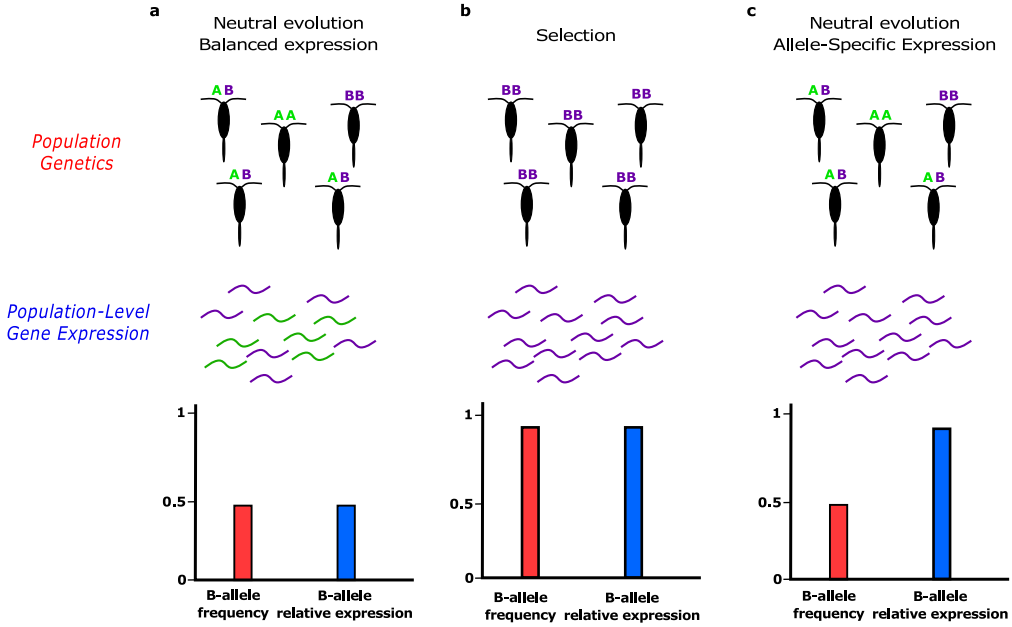

Figure 1: Population genomic and transcriptomic profiles of a biallelic locus in a case of **a,** Neutral evolution and balanced expression; **b,** Selection in favor of the B-allele; **c,** ASE in favor of the B-allele.

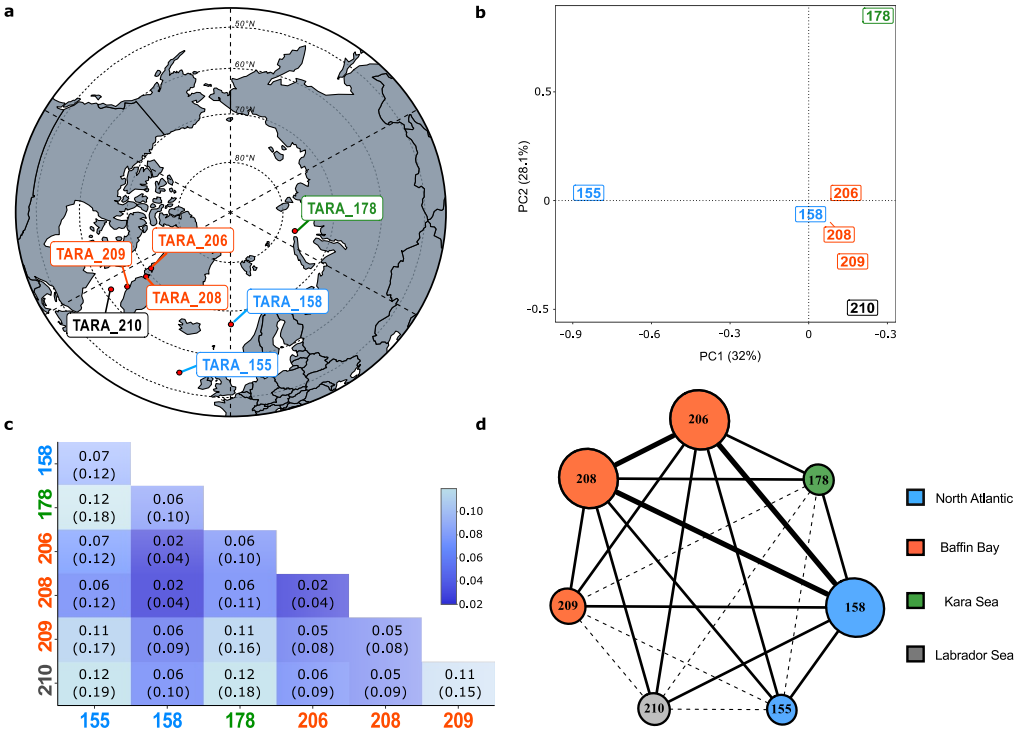

Figure 2: Genomic differentiation of *O. similis* populations from Arctic Seas. **a,** Geographic locations of the seven *Tara* Oceans sampling sites: Northern Atlantic (blue), Kara Sea (green), Baffin Bay (orange) and Labrador Sea (grey). **b,** Principal Component Analysis (PCA) computed by *pcadapt* based on allele frequencies. **c,** Pairwise-F_{ST} matrix. The median (mean) of each pairwise-F_{ST} distribution computed on allele frequencies is indicated. **d,** Graph representing the genomic differentiation of the seven populations of *O. similis*. The nodes represent the populations; their width reflects their centrality in the graph. The edges correspond to the genetic relatedness based on the median pairwise-F_{ST} between each pair of population; 0.02 (large solid line), 0.05 to 0.07 (thin solid line) and 0.11 to 0.12 (dashed line).

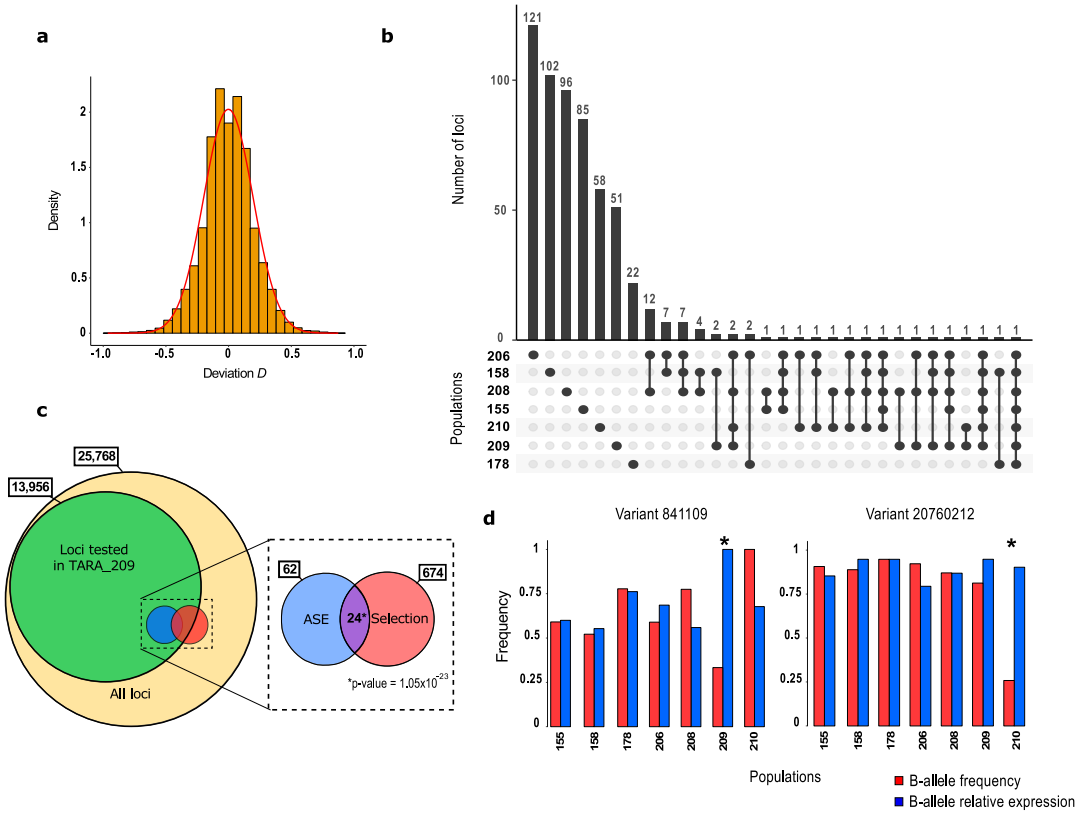

Figure 3: Population allele-specific expression detection and link with natural selection. **a,**The deviation *D* distribution in TARA_209. The red line corresponds to the Gaussian distribution estimated from the data. **b,** Upset plot of the ASE detection in the seven populations. Each bar of the upper plot corresponds to the number of variants under ASE in the population(s) indicated by black dots in the lower plot. **c,** Crossing ASE and Selection. The yellow circle represents the total set of variants. In green, the number of heterozygous variants tested for ASE in TARA_209. In blue and red, the amount of detected variants under ASE in TARA_209 and under selection among the populations respectively. In purple, the intersection comprising variants under ASE in TARA_209 and under selection, with its hypergeometric test p-value. **d,** Metagenomic and metatranscriptomic profiles of variants 841109 and 20760212. Each population is indicated on the x-axis, with the associated B-allele frequency (red) and B-allele relative expression (blue). The frequency is shown on the y-axis. The asterisks mean ASE was detected in the corresponding population.

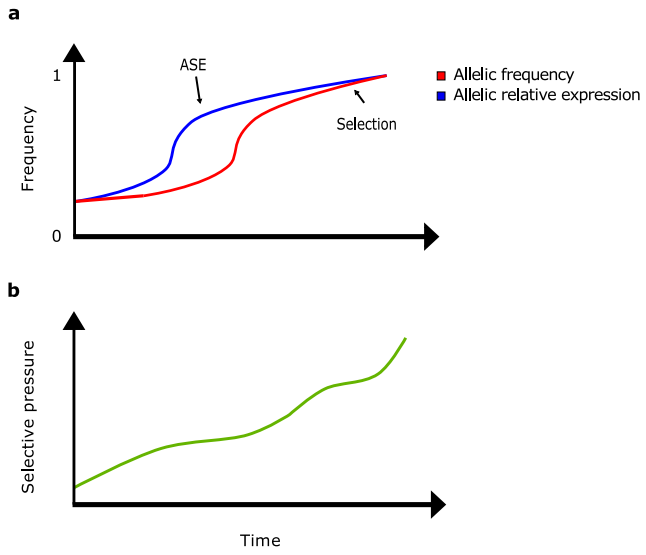

Figure 4: From allele-specific expression to natural selection. **a,** Evolution of allele frequency and allele relative expression over time. **b,** Evolution of selective pressure over time