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Resource

Dissection of the complex genetic basis of craniofacial anomalies using haploid genetics and interspecies hybrids in *Nasonia* wasps

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ABSTRACT

The animal head is a complex structure where numerous sensory, structural and alimentary structures are concentrated and integrated, and its ontogeny requires precise and delicate interactions among genes, cells, and tissues. Thus, it is perhaps unsurprising that craniofacial abnormalities are among the most common birth defects in people, or that these defects have a complex genetic basis involving interactions among multiple loci. Developmental processes that depend on such epistatic interactions become exponentially more difficult to study in diploid organisms as the number of genes involved increases. Here, we present hybrid haploid males of the wasp species pair *Nasonia vitripennis* and *Nasonia giraulti*, which have distinct male head morphologies, as a genetic model of craniofacial development that possesses the genetic advantages of haploidy, along with many powerful genomic tools. Viable, fertile hybrids can be made between the species, and quantitative trail loci related to shape differences have been identified. In addition, a subset of hybrid males show head abnormalities, including clefting at the midline and asymmetries. Crucially, epistatic interactions among multiple loci underlie several developmental differences and defects observed in the F2 hybrid males. Furthermore, we demonstrate an introgression of a chromosomal region from *N. giraulti* into *N. vitripennis* that shows an abnormality in relative eye size, which maps to a region containing a major QTL for this trait. Therefore, the genetic sources of head morphology can, in principle, be identified by positional cloning. Thus, *Nasonia* is well positioned to be a uniquely powerful model invertebrate system with which to probe both development and complex genetics of craniofacial patterning and defects.

1. Introduction

The interpretation of the apparent correlation between form and function in living beings was one of the intellectual advancements that led to formulation of Darwin's theory of evolution, and the concept of homology (Appel, 1987). The problems of how and why forms change in evolution is still timely, and is one of the pillars of the young but maturing field evolutionary developmental biology (Moczek et al., 2015). In regard to evolution, specific forms that are more apt to perform particular functions should be favored, and increase in frequency over time. The developmental basis of changes in form within natural populations are still relatively poorly understood despite the in depth knowledge of developmental mechanisms in few model species. Questions that have not been adequately answered include: How are shape and size regulated at the cellular level during development, and how are these features encoded in the genome? How is symmetry maintained between independently developing halves of bilateral structures, and how is the fusion of multiple tissues into a functioning organ accomplished?

A key innovation in biological form was the cephalization of the early ancestors of the Bilateria. The head is the major structure through which bilateral animals perceive and interact with their environments, and is thus a crucible for interactions between form, function, evolution and development. The concentration of a large number of sensory organs of different types and embryological origins in a relatively small space poses significant developmental challenges, requiring exquisite communication across a complex set of structures, each of which is crucial for the survival

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of the organism (Young et al., 2000).

Consistent with the complexity of head development and rapid evolution of head size and shape in *Homo sapiens*, cranial anomalies are some of the most common birth defects occurring in humans (Stanier and Moore, 2004). A major class of craniofacial defects is oral clefts primarily occurring in the lip and/or palate (CL/P), which occurs in up to 1:300 live births (Wyszynski et al., 1996) and is known to have a complex genetic inheritance pattern involving interactions among several loci (Carter et al., 1982; McKusick, 1994; Prescott et al., 2001; Shields et al., 1981; Wilkie and Morriss-Kay, 2001).

The advent of advanced sequencing techniques has led to the application of large scale approaches, such as genome wide association studies (GWAS) to human craniofacial development ((Dixon et al., 2011; Twigg and Wilkie, 2015). These studies have identified very strong candidate protein coding genes (Leslie et al., 2015; Wolf et al., 2015) and even regulatory regions (Attanasio et al., 2013; Fakhouri et al., 2014) affecting craniofacial development. However, given the large number of genes and epistatic interactions that appear to underlie craniofacial development and disease, the handful of genes with demonstrated roles in humans are likely a tip of a very large iceberg (Hallgrimsson et al., 2014).

Insects can be valuable models systems when attempting to understand complex developmental processes such as craniofacial development. Given that cephalization is a synapomorphy of the Bilateria, a common evolutionary origin unites the vertebrate and invertebrate head, and indeed many of the molecules involved in head patterning and morphogenesis (e.g., Otx/otd genes, BMP, WNT, hedgehog signaling) are highly conserved between vertebrate models (Greene and Pisano, 2010; Hide et al., 2002; Zhang et al., 2002) and *Drosophila* (Royet and Finkelstein, 1997; Shyamala and Bhat, 2002; Stultz et al., 2006; Won et al., 2015). Insects have the added advantage of being easier to maintain as well as having faster generation times than vertebrate model systems, and therefore can be used to reveal candidate genes involved in craniofacial abnormalities and development.

A major contribution to the genetic complexity of craniofacial defects is epistasis-the phenotypic effect of non-additive interactions among alleles at different loci (Lidral and Moreno, 2005). Epistasis has been shown to play an important role in the degenerative craniofacial development of cavefish (Gross et al., 2014), and skull shape trait complexes in crosses between mouse strains (Wolf et al., 2005).

Epistatic interactions among alleles can be difficult to study due to the complexity of the genetics involved. For example, as the number of interacting genes increases, there is an exponentially increasing rarity of progeny homozygous for all of the required alleles. To illustrate, in crosses between two strains differing in a target phenotype in a typical diploid organism, the proportion of F2 offspring (i.e. F1 × F1 parents) revealing a recessive epistatic interaction between two autosomal loci is 1/16, for 3 loci is 1/64, and for 4 loci is 1/256. Backcrossing F1 progeny to either parental strain will not reveal such epistatic interactions among the progeny. The problem is even greater when screening for recessive epistatic interactions within populations (e.g., GWAS), as such approaches are dependent upon allele frequencies among the interacting loci.

In contrast, recessive epistatic interactions are much more readily revealed in organisms with haploid adults, such as the hymenoptera (ants, bees and wasps). These organisms have haplodiploid sex determination, where haploid males develop from unfertilized eggs, and diploid females derive from fertilized eggs. Thus, hymenopteran F2 haploid males will reveal recessive epistatic interactions among 2, 3, and 4 loci at frequencies of 1/4, 1/8, and 1/16 respectively (compare to the respective values of 1/16, 1/64, and 1/256 in diploids). Thus, the statistical power of detecting epistatic interactions is greatly enhanced in such organisms, should they be laboratory tractable.

The parasitoid wasp genus *Nasonia* is emerging as a powerful genetic model system, particularly for complex traits, and developmental and evolutionary genetics (Beukeboom and Desplan, 2003; Lynch, 2015; Werren and Loehlin, 2009). These insects have a short generation time (~two weeks), large family sizes, are easily reared in the laboratory, can be kept under refrigeration for long periods (allowing storage of many strains). They have extensive genomic and transcriptome resources (Werren and Loehlin, 2009; Werren et al., 2010), and genetic tools such as systemic RNA interference (Lynch and Desplan, 2006) and visible and molecular markers to facilitate mapping and cloning of phenotypic traits (Desjardins et al., 2013; Niehuis et al., 2013). A major advantage of *Nasonia* is the ability to perform crosses among closely related interfertile species (Breeuwer and Werren, 1995). This feature permits the mapping and positional cloning of quantitative trait loci (QTL) involved in species differences in development and morphology (Loehlin et al., 2010a; Loehlin and Werren, 2012).

Males of *Nasonia* species differ in cranial shape, with the largest differences being between *Nasonia giraulti* and *N. vitripennis* ((Darling and Werren, 1990), Fig. 1). These two species are separated by approximately 1 million years of independent evolution, and are diverged by ~2 to 3% at the nucleotide level (Werren et al., 2010). In this manuscript, we describe a range of head shapes as well as cranial abnormalities, including abnormal cranial midline furrowing, dorsal–ventral asymmetries, and lateral asymmetries that occur in F2 interspecies hybrid males. Analysis of gene interactions involved in cranial development and cranial abnormalities in hybrid males is greatly facilitated by the ability to more easily detect epistatic interactions in haploids, because at any locus the male is hemizygous for genes from one or the other species, thus removing complications caused by dominance interactions between alleles. This major advantage is enhanced by the powerful genetic toolbox available in *Nasonia* (Lynch, 2015; Werren and Loehlin, 2009).

Since F2 males show considerable variation in head shape, we are able to map major quantitative trait loci (QTL) and epistatic interactions in cranial development. These QTL can be further investigated by introgressing genetic regions containing cranial QTL from one species into the genetic background of the other.

Here we describe the potential of the *Nasonia* system for genetic and molecular analyses of craniofacial development, taking advantage of differences in male head shape between closely related species. We describe the basic system, results of a QTL analysis for head shape revealing major QTL and epistatic interactions, cranial abnormality syndromes in hybrids, the introgression of a head shape locus from *N. giraulti* into *N. vitrpennis*, and future directions using the tools available in Nasonia to reveal gene interactions involved in cranial development.

2. Materials and methods

2.1. Genetic crosses for Quantitative Trait Locus (QTL) analysis

Crossing methods to generate F2 haploid hybrid males for quantitative trait analysis are described in detail in (Gadau et al., 1999, 2002). The same F2 mapping population used in those studies for analysis of hybrid incompatibility loci and wing morphology QTL are used here for mapping of QTL for head morphology. Basically, a cross was initiated between two *Wolbachia* free (Werren, 1997) and highly inbred strains of *N. vitripennis* (AsymCx) and *N. giraulti* (R16A). The *N. giraulti* strain R16A is derived from an introgression of *N. giraulti* nuclear genome in a *N. vitripennis* cytoplasm to avoid nuclear-mitochondrial incompatibilities (Breeuwer and Werren,

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