Title: Phylogeny can Inform Animal Model Development for Both Inherited and Induced Conditions: Duchenne's Muscular Dystrophy (DMD) and Fetal Alcohol Spectrum Disorders (FASD).

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Abstract

The use of animal models in research on human and veterinary diseases and disorders is retracting, though it is likely to remain critical for decades. In light of increasing regulation and expectations of judicious use of animal subjects, we examine the idea that the use of animal models can be guided by phylogenetic relationships and modern evolutionary and cladistic analyses. Given that inherited disorders, and indeed, even the developmental and physiological responses to non-inherited conditions, are subject to evolutionary forces, it follows that the observed differences in model organisms are the products of evolutionary divergence. Understanding that divergence has the potential to elucidate which taxa are most likely to exhibit any given symptom or manifest a reaction in a broadly predictable fashion. We examine two case studies, one the inherited disorder Duchenne's Muscular Dystrophy, and the other an entirely environmentally induced problem, Fetal Alcohol Spectrum Disorder, or Fetal Alcohol Syndrome. Both case studies reveal symptoms are largely congruent with phylogeny, suggesting relatively conservative evolution of developmental pathways. It follows that it is possible to characterize the manifestation of symptoms or dysmorphologies to broad phylogenetic groups. These data can then be used to inform research into possible treatments based on molecular genetic techniques sourced from unaffected taxa or even provide an evolutionary rationale for maximizing ethical decisions in the use and development of animal models in biomedical research. We argue that the technique should become standard practice in the development of animal models.

Keywords: phylogeny, model animals, evolutionary medicine, Duchenne Muscular Dystrophy (DMD), Fetal Alcohol Spectrum Disorders (FASD), Fetal Alcohol Syndrome (FAS)

Introduction

The utility of animal models in biomedical research has been questioned by researchers across a broad range of fields (Johnson et al. 1999, Hegen et al. 2008, Wall & Shani 2008, Nestler & Hyman 2010, Pound, 2018), based on the high failure rate of animal models to successfully predict therapeutic results in target species (Wall & Shani 2008), or based on questions of validity more generally (Nestler & Hyman 2010, Pound 2018). By their very nature, animal models represent evolutionary lineages that have been divergent from their target species (typically our own or those of veterinary interests) for millions to hundreds of millions of years. Thus the premise that responses might yield adequate predictions of the target species is predicated on the conservation of not just genes, but of homologous processes including differential splicing, developmental cascades, and more. Pound and Ritskes-Hoitinga (2018), for example, conclude that failure in animal model development is predominantly due to species-level differences between model organisms and their target species, though Willmann et al (2009) are more optimistic about the value of existing models.

It remains unclear, however, if the predominance of failure is an intrinsic problem or one that's due to the haphazard nature of developing animal models. Indeed, animal models for diseases and genetic disorders have proliferated across taxa so broadly as to seem nearly random. Duchenne's Muscular Dystrophy (DMD), for example, has no fewer than 13 species used as model organisms due in part to such things as accidental discoveries of putatively homologous conditions (e.g., in several breeds of domestic dog).

For Fetal Alcohol Spectrum Disorder (FASD) numerous species have been used in experimental contexts to assess the effects of ethanol exposure during development, ranging from fruit flies to Macaques, and similar situations exist for many other diseases and conditions.

In recent years it has become increasingly clear that the complexity with which developmental and biochemical pathways ultimately produce normal- and dysmorphologies (physical or physiological) in the phenotype is simply unaccounted for in typical model animal research (Richtsmeier et al. 2000), and may be the cause of many failures during clinical trials (Wall & Shani 2008). Much research on dysmorphology is framed in the context of clarifying the roles of individual genes or molecules, though as Reeves and colleagues have argued in the case of Down Syndrome, the ultimate phenotypic expression is likely the result of genetic, environmental, and stochastic influences (Reeves, et al. 2001). More importantly, given that any one gene product may interact with numerous other gene products in the development of phenotype, mutations in one gene product may have wide ranging effects on phenotype (Richtsmeier, et al. 2000), particularly amongst taxa that have been divergent for millions of years, accumulating autapomorphic mutations in interacting gene products. In other words, while a gene of interest (e.g., for Duchenne Muscular Dystrophy) may be homologous amongst model organisms, not only has this gene itself been subject to mutation and divergence for tens of millions of years since its last common ancestor with a model, but all of the genes with which it interacts have been as well. The result likely manifests

itself in the inconsistent phenotypic response to insults seen in animal model studies (see (Richtsmeier et al. 2000).

An ideal heuristic animal model for disease would need to not only possess the causal factor for the disease (e.g., a mutation), but also homologous and unaltered genes for every gene that interacts with that cause. As these conditions are unlikely to be met in any model other than conspecifics, sister taxa logically make the next best possible model organism, which in the case of humans is of course the chimpanzee (Lockwood et al. 2004, Uddin et al. 2004), so presenting numerous ethical concerns and difficulties (Knight 2008). But the choice of appropriate animal model cannot rest upon which organism is most likely to have the most conserved developmental pathways, as the answer would always be the same for each target species, but rather should be one of determining which of the available animal models conserves pathways that are of use in solving particular problems. Willmann et al (2009) provide an exceptional review of several DMD mammalian models that approaches this goal by providing a comparative analysis of symptoms across models, though not explicitly in an evolutionary context. If we can assess to what extent the manifestations found in model organisms are likely to be homologous, then we may better predict which model organisms might make the best positive or negative models for specific traits or symptoms.

In any disease process, the response of the body to the disease is a product of evolution, with the diversity of responses across taxa reflective of the time since the diversification of those taxa. Shared, homologous responses to disease were discussed by Charles Darwin (Darwin 1871) in the context of non-human primate responses to

medications originally developed for humans. In so far as medical treatments for humans result in comparable responses in non-humans, successful treatment indicates that the affected systems evolved their relevant functional structures or physiology before the two taxa diverged. That is, the treatment has the same effect on the non-human system because the system affected is primitive to both taxa. It follows then that divergence in systems affected by disease should logically result in divergence of symptoms or manifestations of the disease and possibly in responses to treatment, likely at least part of the cause of failures in animal testing of medicines.

We explore the feasibility of using the Cladistic methodology known as character optimization, or trait mapping, to examine the evolution of a genetic disorder, Duchenne Muscular Dystrophy (DMD), as well as the non-inherited insult to development known as Fetal Alcohol Spectrum Disorder (FASD)

Duchenne Muscular Dystrophy (DMD)

Muscular dystrophy is a term that encompasses several hereditary diseases characterized primarily by progressive weakness and degeneration of skeletal muscle. The most common form, Duchenne Muscular Dystrophy has been known in humans since the 19th Century, and is known to be inherited as an X-linked recessive trait that affects approximately one in every three thousand males worldwide (Ozawa et al. 1998).

In 1987, the gene's protein product was identified and termed dystrophin ((Hoffman et al. 1987). Briefly, dystrophin provides structure, thus keeping muscle cells intact. Without dystrophin, the membranes become unstable and permeable to substances

that would not ordinarily enter the cells (Ozawa et al. 1998). The onset of DMD is characterized by progressive muscle wasting and increased fat and connective tissue replacement of degenerated muscle fibers in humans. Symptoms typically begin to appear between the ages of two and five, with most affected males confined to a wheelchair by age twelve and rarely living past their twenties (Emery 1998, Willmann et al. 2009).

Fetal Alcohol Spectrum Disorder (FASD)

FASD, an umbrella term commonly used to describe the effects of prenatal ethanol exposure (including the historic term Fetal Alcohol Syndrome), results in an alcohol induced dysmorphology characterized by growth deficiencies, CNS abnormalities, and craniofacial changes, but has no known genetic component (Astley & Clarren 2000). As a result, the specific insult to the developmental system is identical in all model organisms (though variations in dosage have been the subject of many experiments).

As potentially any animal could be used as a model for fetal alcohol exposure, FASD represents a unique case of a common, medically relevant disorder caused solely by an environmentally induced insult to the developmental program. As a result, divergence in symptomatic traits among model organisms can be inferred to reflect otherwise cryptic divergence in the developmental and regulatory genome. Placing animal models into their proper phylogenetic context allows the opportunity for greater understanding of the differential effects of alcohol as a product of the independent

evolution of each lineage used in experimental work. In the present case, animal models used in studying FASD range from the common fruit fly (McClure et al. 2010) to rhesus monkeys (Clarren 1999). Thus the only effects of alcohol on development in *Drosophila* that may be relevant to humans are those that affect homologous genes and developmental pathways that can be traced back nearly 600 million years to the Cambrian explosion.

In order to examine the utility of phylogenetic data in providing insight into both inherited genetic conditions and non-inherited insults during development, we place two well-studied animal model systems in a phylogenetic context for the first time. Consequently, we test the hypotheses that identified symptoms amongst animal models and humans may be homologous at different levels within the phylogeny of animals that may be inferred with graded levels of confidence using phylogenetic methods.

Methods

We conducted a comprehensive search of available databases for literature on DMD and FASD (and related terms) in model animals, ultimately yielding experimental or clinical papers published since 1973, describing the symptoms of these conditions in humans and animal models. We found 47 papers that provide novel descriptions of DMD symptoms in 13 model organisms. While for FASD, we found 42 publications that describe phenotypic manifestations in twelve non-human animals. In addition, symptoms

of each condition in humans were identified in summary publications for DMD and for FASD.

The analysis of character distribution in phylogeny is based on the assumption that the characters are independent of one another (Shaffer 1986, Shaffer & Voss 1996), though that is often not the case (Wilkinson 1995). The use of characters that represent character complexes (also called functional complexes) in phylogenetic analysis has the effect of over-representing what might otherwise be singular underlying causes. Nonetheless, these can be used in phylogenetic analyses when they are treated as single units (Kluge 1989, Lauder 1990). Character complexes may be identified *a priori* based upon knowledge of the biology of the traits, or through analysis of character correlation within a phylogenetic framework (Emerson & Hastings 1998, Wilkinson 1995). The potential for over-representation of characters makes it important to identify possible complexes in any study. In the present case, for example, FASD researchers have cited "reduced intelligence" in humans and model organisms (Streissguth 1986, Meyer & Riley 1986), while others have noted "reduced neocortex size" in some of the same taxa (Valenzuela, et al. 2012). As the former is almost undoubtedly a functional result of the latter, these can reasonably be inferred to represent a biological character complex *a priori*. Similarly, redundancies were eliminated based on descriptions of interpreted as synonymous when they seemed to differ only qualitatively (e.g., cleft lip and cleft palate) or in word choice (e.g., infra-nasal depression and philtrum in FASD). As a result, we reduced the total characters found for DMD to 39 non-redundant symptomatic traits or characters included in subsequent work, while 140 subtly unique descriptions of FASD symptoms were reduced to 57 non-redundant characters. Given the sequence of

developmental events, we regard neurological characters of FASD as likely primary causes of many non-neurological characters, though clear correlations were difficult to substantiate in all cases. As a result, characters were divided into neurological and non-neurological traits and analyzed for biological correlation only within these groupings. Character correlations across these groupings may remain unidentified in the analyses.

A character-taxon matrix was constructed for each test (DMD and FASD), wherein the symptomatic traits were arbitrarily treated as derived character states, wildtype conditions treated as primitive (unaffected), and characters with no data for any given species were treated as unknowns. The amount of data available for each species was not equal; absence of reported symptoms was not presumed to indicate evidence of the absence of the symptoms, with that designation restricted to circumstances where researchers offered specific affirmation that a symptom was absent in a particular taxon. Traits found among derived taxa that could not possibly manifest in more primitive taxa due to absent homologous structures (e.g., forebrain in arthropods, ptosis in fish, etc.) were assigned a value of "absent" in taxa that could not possibly possess them.

Characters were optimized onto abbreviated cladograms of animals (Price et al. 2005, Peterson et al. 2008, Lockwood et al. 2004, Chiari et al. 2012), using Mesquite (Maddison & Maddison 2011) which uses maximum parsimony in character inference for taxa with unknown character states and the AccTran algorithm (favoring reversals over convergences when the choices are equally parsimonious (Farris 1970, Wiley et al. 1991). Root node values were coded as unknown.

Characters absent in the analyses were inferred using maximum parsimony and characterized following the terminology associated with the Extant Phylogenetic Bracket (EPB) method of Witmer (Witmer 1995) for the inference of unpreserved attributes. The use of maximum parsimony often results in all traits inferred to be primitive in studies including taxa with unknown character states (Maddison & Maddison 2011), but fails to make explicit the confidence with which those inferences can be made, a shortcoming that can be corrected following EPB terminology. Consequently, a Level 1 inference is one which is supported by phylogenetic information in bracketing sister taxa for any taxon with an unknown character state (Level 1' in Witmer 1995). That is, traits can be inferred with highest confidence when unambiguously affirmed by phylogeny. Level 2 inferences are those which are supported only by phylogenetic information provided by a single sister taxon, and are thus ambiguous with regard to ancestral character states, clearly a lower level of confidence (Witmer 1995). In the present study, symptomatic traits were optimized to the lowest node where a Level 1 inference could be made. Remaining traits could be inferred with Level 2 confidence, a more speculative inference, in taxa falling outside of the clade where Level 1 inferences were made.

Results and Discussion

Analyses of both DMD and FASD datasets resulted in similar findings, with a minority of symptoms definitively identified as present or absent in animal models for both cases, but providing distributions that allow for Level 1 inferences (highest confidence based on unambiguous phylogeny) for the largest number of unknown

characters (37.9% and 36.2% respectively; see Table 1). This illustrates the fact that a large number of model organisms have been incompletely surveyed for symptoms and suggests they are not being maximally utilized.

Table 1. Summary of Documented and Inferred Symptomatic Traits for DMD and FASD. In both cases, a majority of likely symptoms remain undocumented, but more than 30% of unknown character states are strongly supported by phylogeny in both studies.

	DMD	FASD
Species	13	13
Symptoms	39	57
Total Character States	507	741
Characters Observed	125 (24.7%)	209 (28.2%)
Level 1 Inferences	161 (31.8%)	268 (36.2%)
Level 2 Inferences	221 (43.6%)	264 (35.6%)

DMD Results

Of the nearly 400 inferred character states in the analysis of DMD, 192 can be inferred with the highest, Level 1, confidence based on the fact that the evidence from phylogeny unambiguously suggests that these characters should be present in cases where they remain undocumented. Most of these Level 1 inferred character states map to the level of Mammalia (see Figure 1), suggesting that the underlying genetic programs for these 23 symptoms are actually tied to Mammalian synapomorphies, or shared-derived characters. Five symptoms seem to characterize Amniote, one is inferred for all

Vertebrates, and several are present in all animals. In addition, five may be unique to humans or are untested outside of our species (see Figure 1; Table 2).

Research into DMD has made unusually dense use of the dog as an animal model, with at least ten breeds of dogs represented in the research. Interestingly, different breeds of dog do not all present with identical symptoms, though certainly the breeds have not been equally well studied. The Irish Terrier, for example, presents no evidence of fat infiltration of muscle fibers while the other nine breeds all do (analysis not illustrated). Indeed, the symptom appears to characterize all Amniotes, indicating that the absence of fat infiltration in Irish Terriers is unique (autapomorphic), further suggesting that this breed might present useful genetic data not found in other breeds of dogs, or indeed in any other species.

Within the Mammalia, autosomal inheritance of DMD is unusual, only being found in the mink (Hegreberg et al. 1975, Hegreberg et al. 1976) and hamster (Homburger et al. 1966), while the condition is x-linked amongst other mammals. The gene is also known to be autosomal in both turkeys (Schmitz & Harper 1975) and chickens (Dominguez-Steglich et al. 1990) as well, suggesting that the primitive condition for DMD is an autosomal pattern of inheritance, rather than x-linked, which has profound implications for studies involving these taxa. Indeed, Becker Muscular Dystrophy (BMD) is known to exhibit autosomal recessive inheritance (Ozawa et al. 1998), suggesting that either these animal models may be analogues to DMD, or that DMD itself may have evolved from BMD.

Several other characters (weight gain, gingival overgrowth, ectopic calcifications, and extended viral-induced papillomas) are only known in dogs and known to be absent

in humans. While these are likely of limited clinical interest, phylogenetic evidence for them is equivocal in other model species that might be used for veterinary research.

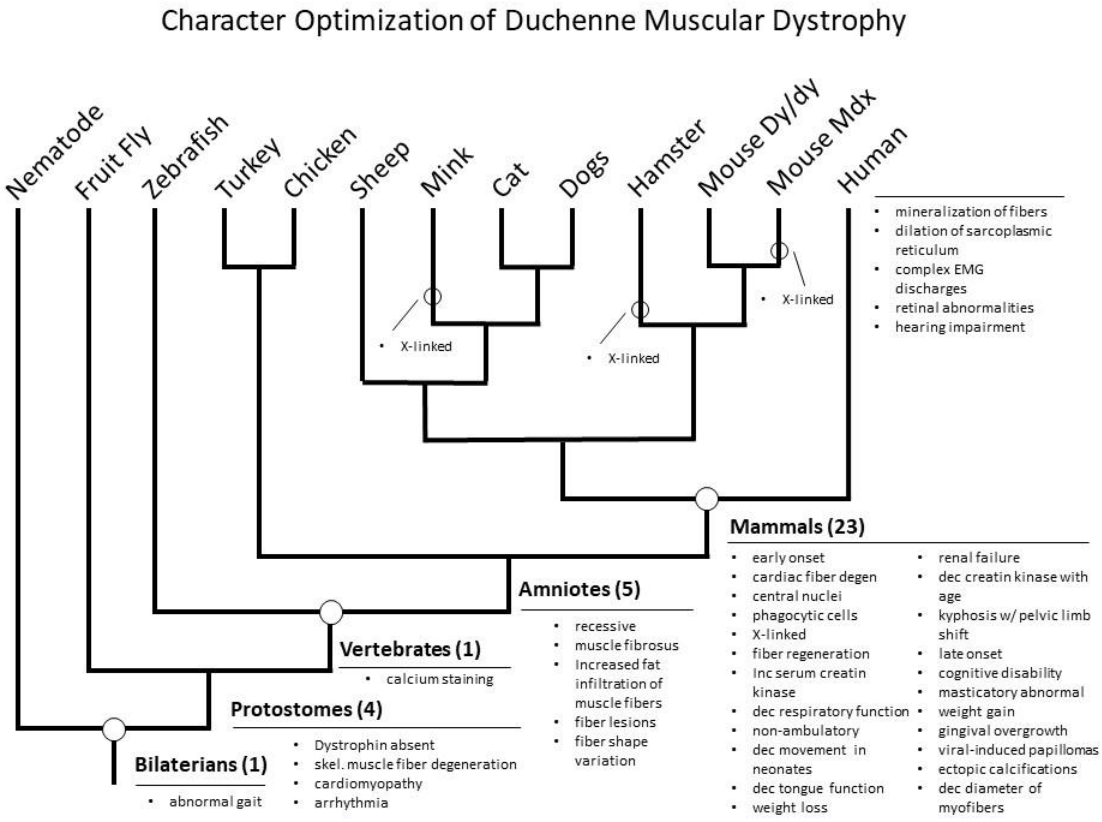

Figure 1. Character Optimization – DMD Symptoms. DMD symptoms are most commonly attributed to Mammals, though there are symptoms which characterize each node within the clade of animals studied. Open circles indicate possible loss of a character.

Table 2-DMD. Documented and inferred character States for Symptomatic Traits among DMD Model Animals. Blue line indicates phylogenetically lower level limits of Level 1 inferences (highest confidence) or Documented symptoms. Key: D = documented symptom; A = documented absent symptom; 1 = Level 1 inference; 2 = Level 2 inference. Dogs are treated as a single case, though the condition has been identified in ten breeds of dog which exhibit variation in some traits (data not shown).

DMD Animal Models	Trait	Clade												
								Mammals						
							Amniotes							
						Vertebrates								
					Animals (Metazoans)									
		Species	Nematode	Fruit Fly	Zebrafish	Turkey	Chicken	Sheep	Mink	Cat	Dogs	Hamster	Mouse (Dy/dy)	Mouse (Mdx)	Human
	Abnormal Gait		D	1	1	1	1	1	D	D	D	1	1	1	D
	Dystrophin absent		2	D	1	1	1	1	1	D	1	1	1	D	D
	Skeletal Muscle Fiber Degen.		2	D	1	D	1	1	D	D	D	D	1	D	D
	Cardiac Failure/ Cardiomyopathy		2	D	1	1	1	1	1	1	D	D	1	1	D
	Arrhythmia		2	D	1	1	1	1	1	1	D	1	1	1	D
	Calcium Staining on Muscle Fibers		2	2	D	1	1	1	1	D	D	1	1	D	D
	Recessive		2	2	2	D	1	1	D	1	D	1	1	1	D
	Muscle Fibrosus		2	2	2	D	1	1	1	1	D	1	1	1	D
	Increased Fat Infiltration of M. Fiber		2	2	2	D	1	1	D	1	D*	1	1	1	D
	Lesions on Muscle Fibers		2	2	2	D	1	1	1	1	D	1	1	1	D
	Cell Fiber Shape Variation		2	2	2	D	1	1	D	1	D	D	1	1	D
	Early Progressive Onset		2	2	2	2	2	D	D	D	D	D	1	1	D
	Cardiac Muscle Fiber Degen.		2	D	2	A	2	1	1	1	D	D	1	1	D
	Centrally located Nuclei in Skel Muscle Fibers		2	2	2	2	2	1	D	1	D	D	1	1	D

	Presence of Phagocytic Cells	2	2	2	2	2	1	D	1	D	1	1	1	D
	X-Linked (non-autosomal)	2	2	2	A	A	1	A	D	D	A	A	D	D
	Skel Muscle Fiber Regeneration	2	2	2	A	2	1	1	1	D	1	1	1	D
	Increased Serum Creatin Kinase	2	2	2	2	2	1	1	D	D	1	1	D	D
	Decreased Respiratory Function	2	2	2	2	2	1	1	D	D	1	1	1	D
	Non-Ambulatory	2	2	2	2	2	1	1	D	D	1	1	1	D
	Decreased M. Movement in Neonates	2	2	2	2	2	1	1	D	D	1	1	1	D
	Reduced Tongue Functionality	2	2	2	2	2	1	1	D	D	1	1	1	D
	Weight Loss	2	2	2	2	2	1	1	D	1	1	1	D	D
	Renal Failure	2	2	2	2	2	1	1	D	1	1	1	1	D
	Decreased Serum Creatin Kinase w/ Age	2	2	2	2	2	1	1	1	D	1	1	1	D
	Kyphosis and Pelvic Limbs Shift Forward	2	2	2	2	2	1	1	1	D	1	1	1	D
	Late Progressive Onset	2	2	2	2	2	1	1	1	D	1	1	1	D
	Cognitive Disability	2	2	2	2	2	1	1	1	D	1	1	D	D
	Masticatory Abnormalities and Salivation	2	2	2	2	2	1	1	1	D	1	1	1	D
	Weight Gain	2	2	2	2	2	2	2	2	D	2	2	2	A
	Gingival Overgrowth	2	2	2	2	2	2	2	2	D	2	2	2	A
	Extended Vira-Induced Papillomas	2	2	2	2	2	2	2	2	D	2	2	2	A
	Ectopic Calcifications	2	2	2	2	2	2	2	2	D	2	2	2	A
	Decreased Diameter of Myofibers	2	2	2	2	2	2	2	2	D	2	2	2	A
	Mineralization of Muscle Fibers	2	2	2	2	2	2	2	2	A	2	2	2	D
	Dilation of Sarcoplasmic Reticulum	2	2	2	2	2	2	2	2	2	2	2	2	D
	Complex EMG Discharges	2	2	2	2	2	2	2	2	2	2	2	2	D
	Retinal Abnormalities	2	2	2	2	2	2	2	2	2	2	2	2	D
	Hearing Impairment	2	2	2	2	2	2	2	2	2	2	2	2	D

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FASD Results

Within the FASD dataset, 531 characters must be inferred, with 268 inferred with Level 1 Confidence. Of these, the majority of symptoms of FASD also seem to characterize Mammalia (24 of 41 characters that can be inferred at Level 1 Confidence). One trait appears to be inherited at the level of Amniotes, while a single additional trait characterizes Vertebrates. Five traits appear to characterize all animals, though they are inferred in 37% of species studied. Finally, three FASD characters known in humans are unknown in any other species (see figure 2; Table 3).

The available literature on FASD is strongly biased in favor of human medicine and mouse models, with these organisms boasting the highest numbers of total documented symptoms (47 and 42 respectively), with ferrets and dogs having the fewest (2 each). A single undocumented trait can be inferred with Level 1 confidence for humans, a reduction in cranial neural crest cells, a trait that cannot be readily studied experimentally. Highest confidence inferences in other species range from none (Fruit fly) to more than 30 previously undocumented symptomatic traits in a number of model species (see Figures 2; Table 3).

Highly fundamental symptoms of FASD seem to characterize all or nearly all animals, including microcephaly, reduced body size, and reduced viability. Vertebrates appear to be characterized by a reduction in cranial neural crest cells (Cartwright & Smith, 1995), musculoskeletal, reproductive, circulatory defects, as well as a large number of facial dysmorphologies Mammals, by far the most thoroughly studied, are characterized by a large number of neurological and non-neurological symptomatic traits,

with non-neurological symptomatic traits including many of the classic facial dysmorphologies as well as non-cranial defects. Within Mammals, sheep, guinea pigs, mice, macaques, and humans all present autapomorphic (unique) conditions not seen in other taxa, while primates also present two synapomorphic (shared-derived) traits, reduction in brain stem development and epicanthic folds (Lebel et al. 2011, Miller 2007), perhaps the most easily recognized phenotype of FASD in some human populations.

A reduction in cranial neural crest cells correlates with a large number of facial dysmorphologies (see Fig. 1 and 2), characterizing all vertebrates. Given the causative role of neural crest cells in the development of numerous facial structures (Johnston 1966), it seems likely that these traits may ultimately prove to be a character complex, with the effects on skeletal and soft-tissue structures solely the result of cranial neural crest cell reduction. Indeed, the fact that shortened noses are known in chicken (Ahlgren et al. 2002) and consequently appear to characterize amniotes, suggests that other craniofacial dysmorphologies may simply be more readily identified in mammals but actually characterize larger clades. Other vertebrate characters include primarily mesodermally derived structures, indicating that additional cell lines or developmental pathways are affected beyond neural crest cells.

Autapomorphic traits among mammals include a dramatic craniofacial defects such as aglossia (O'Leary-Moore et al. 2011) as well as comparatively minor defects (decreased peripheral arterial resistance (Ramadoss et al. 2006, Parnell et al. 2007). These traits suggest divergence in developmental programming that leaves some taxa more vulnerable to alcohol insults than others. Note, however, that in the present study

we did not control for dosage differences across animal model groups, leaving open the possibility that these symptoms could simply occur at different threshold levels than seen in other taxa, a feature that has been shown to affect embryo viability (Becker & Shibley 1998, Carvan & Loucks 2004, Oxendine et al. 2006).

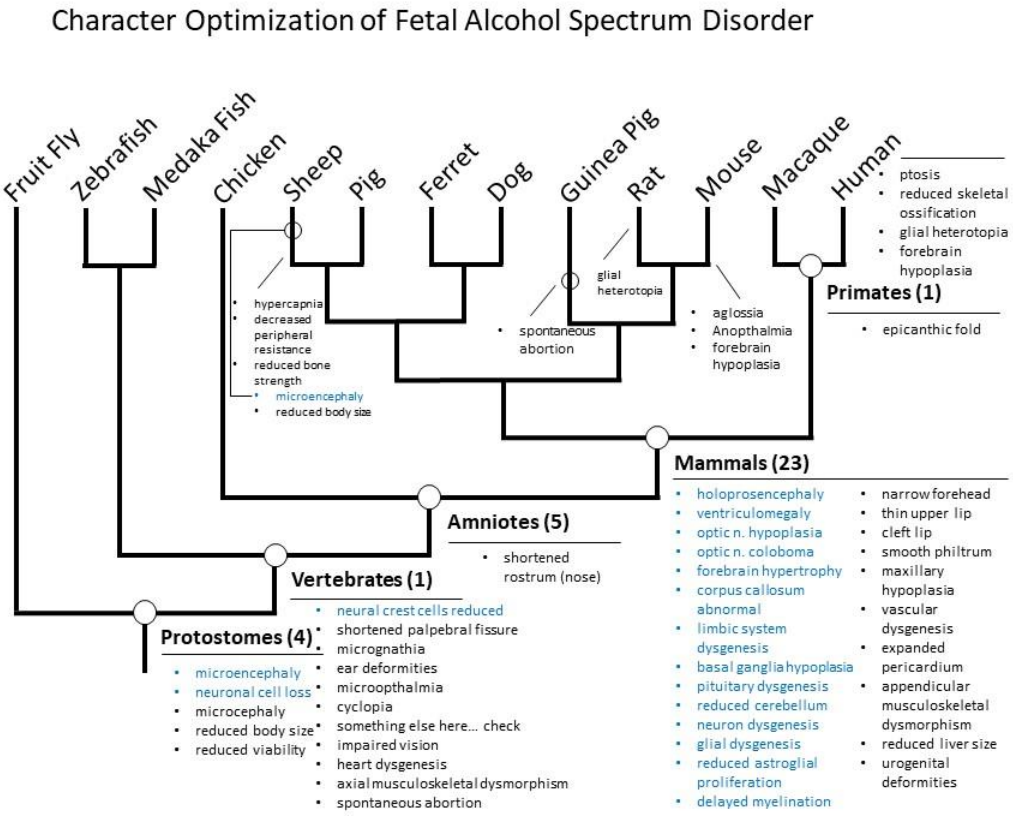

Figure 2. Character Optimization – FASD Symptoms. FASD symptoms are most commonly attributed to Mammals, though there are symptoms which characterize each node within the clade of animals studied. Open circles indicate loss of a character. Neurological characters are listed in blue.

Table 3-FASD. Documented and Inferred Character States for Symptomatic Traits Among Model FASD Animals. Blue line indicates phylogenetically lower level limits of Level 1 inferences (highest confidence) or Documented symptoms. Key: D = documented symptom; A = documented absent symptom; 1 = Level 1 inference; 2 = Level 2 inference.

Subdivision (Neuro / Non-Neuro)	Trait	Clade				Animals (Metazoans)	Vertebrates	Amniotes	Mammals					
		Species	Fruit Fly	Zebra Fish	Medaka Fish	Chicken	Sheep	Pig	Ferret	Dog	Guinea Pig	Rat	Mouse	Macaque	Human
Neurological Symptomatic Traits	Microencephaly		D	1	1	D	A	2	2	2	D	D	D	D	D
	Neuronal Cell Loss		D	1	D	1	1	1	1	1	1	D	D	D	D
	Cranial Neural Crest Cell Reduction		A	D	1	D	1	1	1	1	1	1	D	1	1
	Holoprosencephaly		A	2	2	2	1	1	1	1	1	1	D	1	D
	Ventriculomegaly		A	2	2	2	1	1	1	1	1	1	D	1	D
	Optic N. Hypoplasia		A	2	2	2	1	1	1	1	1	D	D	1	D
	Optic N. Coloboma		A	2	2	2	1	1	1	1	1	1	D	1	D
	Forebrain Hypertrophy		A	2	2	2	1	1	1	1	1	1	D	D	D
	Corpus Callosum Abnormalities		A	2	2	2	1	1	1	1	1	1	D	D	D
	Limbic System Dysgenesis		A	2	2	2	1	1	1	1	D	D	D	D	D
	Basal Ganglia Hypoplasia		A	2	2	2	1	1	1	1	1	1	D	1	D
	Pituitary Dysgenesis		A	2	2	2	1	1	1	1	1	1	D	1	D
	Reduced Cerebellum		A	2	2	2	D	1	1	1	1	1	D	D	D
	Neuron Dysgenesis		2	2	2	2	1	1	1	1	1	D	D	1	D
	Glial Dysgenesis		2	2	2	2	1	1	1	1	1	D	1	1	D
	Reduced Astroglial Proliferation		2	2	2	2	1	1	1	1	1	D	1	1	D
	Delayed Myelination		2	2	2	2	1	1	1	1	1	D	1	1	D
	Glial Neuronal Heterotopia		2	2	2	2	2	2	2	2	2	D	A	A	D
	Anencephaly		2	2	2	2	2	2	2	2	2	2	2	2	D
	Exencephaly		A	2	2	2	2	2	2	2	2	2	D	2	2
	Forebrain Hypoplasia		A	2	2	2	2	2	2	2	2	2	D	A	D
	Reduced Brain Stem		A	2	2	2	2	2	2	2	2	2	2	D	D
Non-	Microcephaly		D	D	D	D	1	1	1	D	D	D	D	D	D

	Reduced Body Size	D	D	D	D	A	D	D	D	D	D	D	D	D
	Reduced Viability	D	D	1	D	1	D	1	1	1	1	D	1	D
	Shortened Palpebral Fissure Length	A	D	1	1	1	1	1	1	1	1	D	D	D
	Micrognathia	2	D	1	1	1	1	1	1	1	1	D	D	D
	Ear Deformities	A	D	1	1	1	1	1	1	1	1	D	1	D
	Microopthalmia	A	D	1	1	1	1	1	1	1	D	D	D	D
	Cyclopia	2	D	1	1	1	1	1	1	1	1	D	1	D
	Eye Deformities	2	D	1	D	1	1	1	1	1	1	D	1	D
	Impaired Visual Function	2	D	1	1	1	1	D	1	1	1	D	1	D
	Heart Dysgenesis	2	D	1	1	1	1	1	1	1	1	D	1	D
	Axial Musculoskel Dysmorphisms	A	D	1	1	1	1	1	1	1	D	1	1	D
	Spontaneous Abortion	2	2	D	2	1	D	1	1	A	2	D	1	D
	Short Rostrum (Nose)	A	2	2	D	1	1	1	1	1	1	D	D	D
	Narrow Forehead	2	2	2	2	1	1	1	1	1	1	D	D	D
	Thin Upper Lip	A	2	2	2	1	1	1	1	1	1	D	D	D
	Cleft Lip	A	2	2	2	1	1	1	1	1	1	D	1	D
	Smooth Philtrum	A	2	2	2	1	1	1	1	1	1	D	D	D
	Maxillary Hypoplasia	2	2	2	2	1	1	1	1	1	1	D	1	D
	Vascular Dysgenesis	2	2	2	2	1	1	1	1	1	1	D	1	D
	Overly Expanded Pericardium	A	2	2	2	1	1	1	1	1	1	D	1	D
	Append. Musculoskel. Deformities	2	2	2	2	D	1	1	1	1	1	D	1	D
	Reduced Liver Size	2	2	2	2	1	1	1	1	1	D	1	1	D
	Urogeneital Deformities	2	2	2	2	1	1	1	1	1	1	D	1	D
	Scaphocephaly	A	2	2	2	2	2	2	2	2	2	2	D	2
	Epicanthic Folds	A	A	A	2	2	2	2	2	2	2	2	D	D
	Ptosis	A	A	A	2	2	2	2	2	2	2	2	2	D
	Choanal Atresia	A	A	A	2	2	2	2	2	2	2	D	2	2
	Aglossia	A	2	2	2	2	2	2	2	2	2	D	2	2
	Anopthalmia	2	2	2	2	2	2	2	2	2	2	D	2	2
	Decreased Peripheral Resistance	2	2	2	2	D	2	2	2	2	2	2	2	2
	Hypercapnia	2	2	2	2	D	2	2	2	2	2	2	2	2
	Pericardial Edema	A	D	2	2	2	2	2	2	2	2	2	2	2
	Reduced Skeletal Ossification	A	2	2	2	2	2	2	2	2	2	2	2	D
	Reduced Bone Strength	A	2	2	2	D	2	2	2	2	2	2	2	2

1

Overall, the application of cladistic character optimization to the study of animal models identifies phenomena that are characteristic of evolutionary studies more generally, features such as evolutionary convergences, reversals, synapomorphic and autapomorphic characters, only in the context of disease or inherited disorders. The utility of these data is largely unexplored, though there is a long-standing discussion about the failure rate of therapies developed with animal models that has been attributed to species-specific (i.e., evolutionary) differences between model and target (Pound 2018). This research suggests that some symptoms manifest due to homology in model animals, which may improve response to treatments.

Evolutionary reversals and convergences represent indications of evolutionary solutions to symptoms in both DMD and FASD. For example, sheep fail to exhibit reduced brain size (microencephaly) and reduced body size; (Ramadoss et al. 2006), otherwise both are synapomoprhies for all animals, potentially providing the opportunity to develop negative animal models for given symptoms (see Rand 2008). Similarly, guinea pigs apparently lack spontaneous abortion (Gibson et al. 2000) otherwise demonstrated across Vertebrates (see Table 1, Fig. 2). Likewise, macaques exhibit conditions apparently homologous to the human reaction, except that they do not experience forebrain hypoplasia (Lebel et al. 2011, Sulik et al. 1981), nor do they exhibit glial neuronal heterotopia (Guerri et al. 2001, Stromland & Dolores 2002). Assuming these reports are accurate, macaques then represent a natural negative model for at least these symptoms of fetal alcohol exposure. A better understanding of the biology of these reversals may prove beneficial to the development of therapeutic techniques.

Convergent evolution is also apparent among animal models examined to date. Specifically, humans and mice may both exhibit forebrain hypoplasia in FASD (Lebel et al. 2011, Sulik et al. 1981), while humans and rats exhibit glial heterotopia (Guerri et al. 2001,

Stromland & Dolores 2002). Both conditions are documented as absent in macaques and other close mammalian relatives (see Table 1).

Autapomorphic responses in humans may not actually be unique. Descriptions of the absence of characters in model animals are irregular at best, with few papers providing clear affirmations of absence in either DMD or FASD. In DMD, for example, hearing impairment is only known to characterize humans, but we found no evidence that this was examined in any model species. Similarly, studies of FASD in model animals vary in both timing and dosage of ethanol exposure, and resulting symptoms which may appear unique, may only be unique because of specific experimental conditions carried out in those studies. Likewise, apparently unique characters in humans may either be unique to our species or may be exposure to additional non-alcoholic teratogens, such as illicit drugs and tobacco (Abel 1995, Becker & Shibley 1998), which cannot be controlled in human studies, and suggests caution in confidence levels of these traits.

The use of parsimony in reconstruction of ancestral character states and infer undocumented symptoms in model organisms is only one possible tool for phylogenetic inference. Indeed, there is significant recent debate on whether parsimony is the most suitable option for such inferences (Wright & Hillis 2014, Sansom et al. 2018) with though it seems that parsimony is appropriate for morphological characters (Goloboff et al. 2018). In any case, a similar analysis of molecular or other data from model organisms making use of Bayesian or other probability-based models of character evolution could also be expected to provide similar insights. The use of a phylogenetically rigorous comparative method has long been shown to provide insights that ahistorical approaches to biology fail to identify (Harvey & Pagel, 1991) and we recommend any approach to animal model evaluation that includes evolutionary history.

Conclusions

While excellent reviews of model animals exist for many biomedical issues, (Driscoll et al. 1990, Pound 2018, Collins & Morgan 2003, Willmann et al. 2009), none have previously examined symptoms or traits for phylogenetic correlations. Placing symptoms of diseases and disorders into their proper evolutionary (phylogenetic) context clarifies existing shortcomings in our understanding of the diversification of developmental systems affected. Understanding this diversification can elucidate both potential advantages and disadvantages among possible animal models and may provide insight into developmental pathways or disease progression.

The case studies used here were chosen to test the utility of studying an inherited disorder (DMD) as well as the responses to a developmental insult, exposure to ethanol (FASD). The data for DMD suggest that this method of analysis provides evidence of evolution of the symptoms of DMD across a diverse array of species. We suggest that the symptoms may reflect the large number of systems with which the dystrophin molecule interacts, and that the differential symptoms are reflective of evolutionary diversification in those systems. The case of fetal alcohol exposure was chosen because it represents an identical insult to development in all species. As a result, deviations in the response to that insult are necessarily reflective of divergence in developmental pathways amongst animals studied. This may be quite similar to a case where a mutation in one gene like dystrophin can have wide ranging effects because of the numerous interactions of the dystrophin molecule. While it is unsurprising that the majority of traits map to the common ancestor of mammals in both cases, it is nonetheless highly informative to be able to infer unpreserved symptoms in species that have not been examined for

all traits. Indeed, this approach has identified hundreds of untested hypotheses within animal models for both DMD and FASD and could be applied to numerous other diseases and conditions. The result of such an application would be improved, systematic analysis of animal models and the identification of innumerable untested hypotheses, which together may increase the translation of model animal research. We look forward to improvements in systematic collection of animal model symptom data that may confirm or refute the inferences articulated above.

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