Supplemental Results

Many marginal variants in female and sex-average epistasis analyses map to pleiotropic genes, continued

Beadex (*Bx*; human homolog *LMO1*) is known to interact with *apterous* (*ap*) in the wing discs, where *ap* contributes to wing morphogenesis and neuronal pathfinding (MILAN *et al.* 1998). *Bx* is also involved in dorsoventral patterning of the wing blade, the same wing blade axis other studies hypothesize as a main driver of morphological variation (MUNOZ-MUNOZ *et al.* 2016; PITCHERS *et al.* 2019). *CG9171* (human homolog *B4GAT1*) is a glucuronosyltransferase predicted to localize to the Golgi and perform O-linked mannosylation. It is known to affect flight performance (SCHNORRER *et al.* 2010) and has a putative role in muscular dystrophy (BUYSEE *et al.* 2013). Similarly, *CG15651* (human homolog FKRP) is also predicted to affect O-linked mannosylation in the Golgi complex and is linked to muscular dystrophy as well (BROCKINGTON *et al.* 2001). The marginal variant associated with *CG15651* also overlapped with *CG9313*, an axonemal outer arm dynein intermediate chain involved in sperm mobility and audiosensation in the Johnston's organ (ZUR LAGE *et al.* 2019). Finally, a marginal variant mapped to *javelin-like* (*jvl*), important for actin and microtubule organization, mechanosensing macrochaete formation, muscle formation in flight, and oogenesis (TILNEY *et al.* 2003; SCHNORRER *et al.* 2010). The corresponding and significant epistatic interactions (Table S9), like these marginal genes, have annotations for wing morphology, muscle development, neural circuit assembly and neuronal function, and interestingly, sex-related behaviors and sex-specific tissues.

Epistatic interactions associating with the sex-difference phenotype, continued

As a trans-regulator, affecting the expression profiles of downstream genes, *ush* had many significant interactions. These include interactions with genes annotated for gravitaxis and locomotion (*CASK, CG34353, dnc, InR, ITP, mid1, Neto, nmo, Syn2, unc-104),* sensory organ development (*aPKC, CG9313, Dl, dpr1, dpr9, dpr10, fry, fz, Gyc88E, mew, mib, rdgA*), dendrite morphogenesis and self-avoidance (*acj6, CadN, Cbp53E, Cont, cv-c, fru, fry, hdc, mAChR-B, Mob2, mtt, Nedd4, Prosap, pum, shn, Tm1, unc-104*), and learning and memory (*aPKC, CASK, cher, dnc, gom, klg, lillo, Mob2, Nep4, Rkg1, pum, scrib, sNPF-R, teq*). There were also epistatic interactions with genes annotated for courtship behaviors (*Btk29A, CASK, dnc, fru, gom, Rgk1*). In particular, *fruitless* (*fru*) was identified in the previous flight performance screen as an epistatic interactor with *ppk23* (SPIERER *et al.* 2020) where it held the largest share of epistatic interactions for a single gene. *Fruitless* is also a transcription factor that patterns sex-specific, neural circuits that connect leg and wing chaete (functioning as contact chemosensors for pheromone detection) to the thoracic ganglion (flight control center) and brain, and out along motor neurons to the flight musculature (for visual flagging and courtship song) (YU *et al.* 2010; PAVLOU AND GOODWIN 2013; SHIRANGI *et al.* 2016).