In *Drosophila* successful mating depends of male activity and female receptivity (Manning, 1961; Spieth, 1968). Table 2 shows in both monomorphic and polymorphic strains of *D. ananassae* middle aged females mated faster, copulated longer than young or old aged females. This suggests that middle age females were more receptive than young or old aged females. This supports earlier studies of *Drosophila* suggesting that females which mate faster and longer are more receptive than the females which mate slower and copulate shorter. This is because mating latency (time taken for initiation of mating) and copulation duration (time between initiation of copulation to termination of each pair) are good estimates of female receptivity (Spieth and Ringo, 1981; Hegde and Krishna, 1997).

In turn males of *D. ananassae* showed greater courtship activities to middle aged females compared to young or old aged females suggesting influence of female age on male courtship activities in too (Table 2). Through these courtship activities males of *D. ananassae* not only convey chemical, auditory, visual signals to middle aged females better and try to convince the middle aged female faster for mating than young or old aged females. This agrees with earlier studies of *Drosophila* that males which perform greater courtship activities are better mates and obtained greater mating success than those males which do not show high level of courtship activities (Hegde and Krishna, 1997).

Thus these studies suggest that males of *D. ananassae* exercised mate choice to obtain direct fecundity benefits, and we also found occurrence of age specific reproductive success in females of *D. ananassae* which is independent from influence of inversion system.

Acknowledgment: The authors are grateful to the Department of Studies in Zoology and Principal, Yuvaraja's College, University of Mysore, for providing facilities.

References: Amundsen, T., and E. Forsgren 2001, Proc. Natl. Acad. Sci., USA 98: 13155-13160; Bock, L.R., and M.R. Wheeler 1972, Univ. Tex. Publ. 7213: 1-102; Bonduriansky, R., 2001, Biol. Rev. 76: 305-339; Branquart, E., and J.L. Hemptinne 2000, Eur. J. Entomol. 97: 165-170; Burley, N., 1977, Proc. Natl. Acad. Sci., USA 74: 3476-3479; Byrne, P.G., and W.R. Rice 2006, Proc. Roy. Soc. Lond. Ser. B 273: 917-922; Chenoweth, S.F., P. Doughty, and H. Kokko 2006, Ecol. Lett. 9: 179-184; Dewsbury, D.A., 1982, Am. Nat. 119: 601–610; Elens, A., and J.M. Wattiaux 1964, Dros. Inf. Serv. 39: 118-119; Galvani, A., and R.A. Johnstone 1998, Behav. Ecol. Sociobiol. 44: 161–168; Gowaty, P.A., R. Steinichen, and W.W. Anderson 2003, Evolution 57: 2037-2045; Hegde, S.N., and M.S. Krishna 1997, Anim. Behav. 54**:** 419-426; Johnstone, R.A., J.D. Reynolds, and J.C. Deutsch 1996, Evolution 50: 1382-1391; Jones, I.L., and F.M. Hunter 1993, Nature (London) 362: 238-239; Katvala, M., and A. Kaitala 2001, Anim. Behav. 62: 133–137; Kokko, H., and R.A. Johnstone 2002, Nature 362: 238-239; Kokko, H., and P. Monaghan 2001, Ecol. Lett. 4: 159-165; Manning, A., 1961, Anim. Behav. 9: 82-92; Milinski, M., 2001, In: *Model Systems in Behavioral Ecology: Integrating Conceptual, Theoretical and Empirical Approaches.* (Dugatkin, L.A., ed.), pp. 245-264, Princeton University Press, Princeton; Owens, I.P.F., and D.B.A. Thompson 1994, Proc. Roy. Soc. Lond. B 258: 93-99; Parker, G.A., 1983, J. Theor. Biol. 101: 619–648; Prathibha, M., and M.S. Krishna 2010, Zoological Studies 49 (6): 806-815; Robertson, F.W., 1957, J. Genet. 55: 428-443; Servedio, M.R., and R. Lande 2006, Evolution 60: 674-685; Singh, B.N., 1985a, Nucleus 28: 169-176; Singh, B.N., 1985b, Theoret. Appl. Genet. 69: 437-441; Speith, H.T., 1968, Studies in Genetics, Univ. Texas Publ. 4: 319-333; Spieth, H.T., 1952, Bull. Amer. Mus. Natur. Hist. 99**:** 395-474; Spieth, H.T., and J.M. Ringo 1983, In: *The Genetics and Biology of* Drosophila. Vol. 3c. (Ashburner, M., H.L. Carson, and J.N. Thompson, jr., eds.), pp. 223-284, Academic Press, London, England.

Genetic evidence for differential activities of $G\alpha_O$ isoforms in *Drosophila melanogaster*.

Di Gioacchino, V., T. Barwell, and L. Seroude*. Department of Biology, Queen's University, Kingston, Ontario, Canada K7L 3N6; *Corresponding author, e-mail: seroudel@queensu.ca.

$G\alpha_o47A$ encodes two isoforms of the $G\alpha_O$ subunit ($G\alpha_O1$ and $G\alpha_O2$) in *Drosophila melanogaster* (de Sousa *et al.*, 1989; Schmidt *et al.*, 1989; Thambi *et al.*, 1989; Yoon *et al.*, 1989). These two isoforms are

98% identical and differ from each other by only 7 single residue changes within the 21 N-terminal amino acids (Figure 1). Although this region is evolutionarily conserved, it is amorphous (Slep *et al.*, 2008); hence, molecular modeling cannot predict what structural consequences these differences might have. Studies on this gene have only used genetic constructs encoding one of the isoforms ($G\alpha_O2$, referred to as cDNA class II in Yoon *et al.* (1989)). We have cloned the open reading frames encoding $G\alpha_O2$ and $G\alpha_O1$ (class I cDNA in Yoon *et al.* (1989)) into the pINDY5 *P* element injection vector (Kazemi-Esfarjani and Benzer, 2000) under the control of a UAS promoter (Di Gioacchino, 2014). Each construct was injected into w^{1118} embryos to generate 8 and 12 strains with independent insertions for $G\alpha_O1$ and $G\alpha_O2$, respectively.

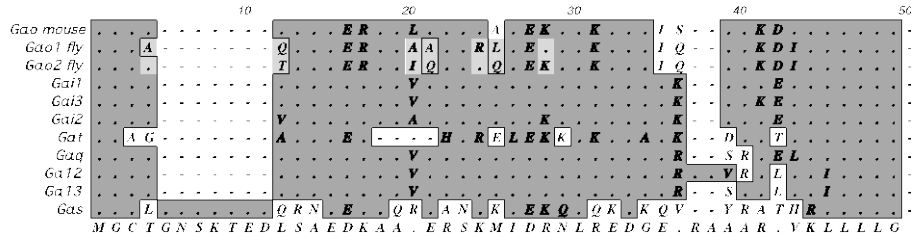

Figure 1. Sequence alignment of heterotrimeric Gα subunit N-termini using methods of Slep *et al.* (2008) and *D. melanogaster* sequences from Yoon *et al.* (1989). Dark grey indicates similar residues, dots indicate identical residues, and light grey highlights the 7 differences between the *Drosophila* isoforms. The last 6 residues correspond to the first ß-sheet motif.

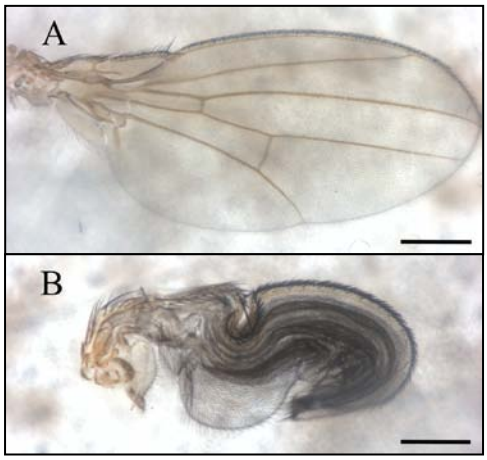

Figure 2. (A) A wild-type wing (32× magnification, scale bar = 0.4 mm), and (B) a crumpled wing (64× magnification, scale bar = 0.2 mm) from two DJ858/+; VD131/+ individuals.

Driven by the DJ858 GAL4 enhancer trap (Seroude, 2002), expression of *UAS-Gα$_O$2* caused a crumpled wing phenotype (Figure 2). This phenotype has been previously observed when $G\alpha_O2$ overexpression was driven by *MS1096-GAL4* (Katanayeva, *et al.*, 2010). Crumpled wings result from the failure of wing maturation, caused by a $G\alpha_O$ subunit competing with and antagonizing the function of a $G\alpha_S$ subunit in a heterotrimer with Gβ13F and Gγ1. DJ858-driven expression of *UAS-Gα$_O$2* caused the crumpled wing phenotype with 7 of the 12 insertions, at varying levels of penetrance (Figure 3). In contrast, only 1 of 8 independent *UAS-Gα$_O$1* insertions caused this phenotype. Unless the insertional biases of the two *P* elements are fundamentally different from each other, the range of expression levels for each construct should be comparable. This suggests that the two isoforms have different biological activities.

One of the DJ858/+;$G\alpha_O2$/+ genotypes (VD134) also caused a noticeable level of pupal lethality and only a few escapers could be recovered. Obvious lethality was not observed with any of the DJ858/+;$G\alpha_O1$/+ genotypes. This observation suggests that $G\alpha_O2$ can cause lethality in addition to the crumpled wing phenotype. Attempts to increase the copy number of both transgenes were unsuccessful since DJ858;$G\alpha_O1$ or DJ858;$G\alpha_O2$ flies exhibit a high rate of lethality. Therefore, increased expression of *UAS-Gα$_O$1* transgenes can also be lethal to the flies.

It was also found that the presence of the TM3, Sb balancer exacerbated the crumpled wing phenotype caused by the expression of *UAS-Gα$_O$2*. $G\alpha_O2$ flies carrying the balancer showed a noticeably higher frequency of the crumpled wing phenotype compared to flies that had a wild-type third chromosome (Figure 4). The addition of the TM3, Sb balancer in a subset of *UAS-Gα$_O$1* genotypes did not induce the crumpled wing phenotype. In addition, there were significantly fewer $G\alpha_O2$ progeny carrying the balancer than expected. These observations indicate that the TM3, Sb chromosome carries one or more mutations that are epistatic to $G\alpha_O2$ expression with respect to both the crumpled wing phenotype and lethality.

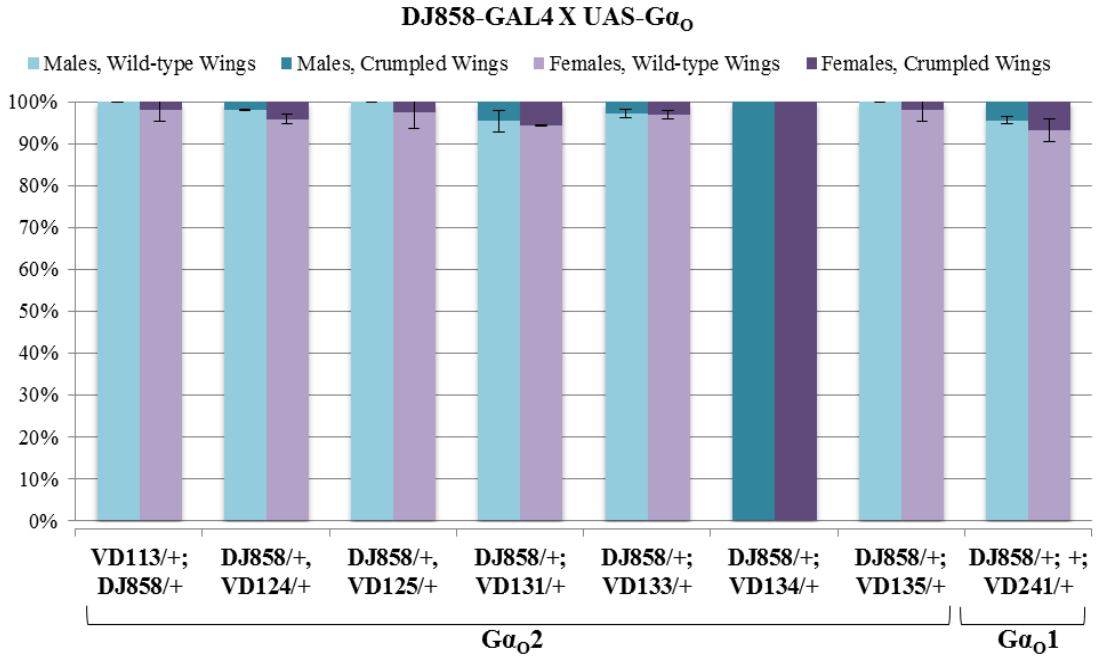

Figure 3. Frequency of the crumpled wing phenotype in progeny carrying *DJ858-GAL4* and either *UAS-Gα$_O$2* or *UAS-Gα$_O$1*. Strains that did not display the phenotype were omitted. Average of two independent experiments; an average of 90 individuals was obtained per replicate, with the exception of VD134 (replicate 1: 4, replicate 2: 3).

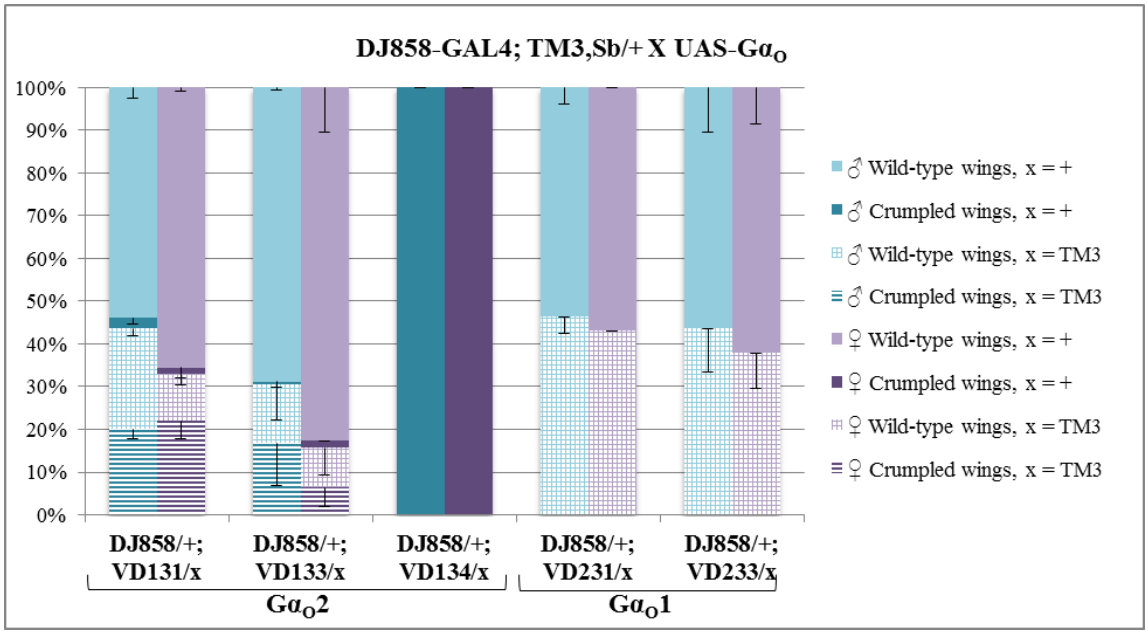

Figure 4. Gα$_O$-TM3,Sb epistasis: frequencies of the wing and bristle phenotypes in progeny carrying *DJ858-GAL4*, *UAS-Gα$_O$2* or *UAS-Gα$_O$1*, and the TM3,Sb balancer or a wild-type third chromosome ("x"). Average of two independent experiments; an average of 110 individuals was obtained per replicate, with the exception of VD134 (replicate 1: 1, replicate 2: 3). χ^2 tests for progeny with and without TM3, Sb: VD131 p♂ > 0.05, p♀ < 0.001; VD133 p♂ < 0.005, p♀ < 0.001; VD231 p♂ > 0.05, p♀ > 0.05; VD233 rep. 1 p♂ < 0.05, p♀ < 0.05, rep. 2 p♂ > 0.05, p♀ > 0.05.

In order to function properly, Gα subunits need to interact with G-protein coupled receptors, β and γ subunits, GTP nucleotides and regulators of G-protein signalling (RGS). Many of the domains that play a key role in these interactions have been well characterized and are evolutionarily conserved (Slep *et al.*, 2008). It has been demonstrated that the 21 N-terminal residues of an α subunit are involved in its interaction with the βγ heterodimer (Navon and Fung, 1987). However, the specific residues that are critical for that interaction have not yet been identified. The presence of two $G\alpha_O$ isoforms in *Drosophila* provides a unique opportunity to investigate structure-function relationships in the N-terminus. Preliminary findings in this report indicate that these two isoforms are not biologically equivalent. Overexpression of both isoforms can cause crumpled wings and lethality, although to a different degree. Furthermore, only $G\alpha_O2$ exhibits epistatic interaction with TM3, Sb. Therefore, these differential biological effects are likely due to one or more of the 7 single residue changes that differentiate $G\alpha_O2$ from $G\alpha_O1$. However, it remains to be shown that these changes are directly involved in interactions with βγ subunits.

It is worth noting that one of the TM3 break points (76C - 71C) is located at the same cytological position as *Gβ76C* (St. Pierre *et al.*, 2014), bringing forth the possibility that TM3 does not carry a wild-type *Gβ76C* allele. It is tempting to speculate that $G\alpha_O2$ and Gβ76C compose the same G-protein. A mutation in *Gβ76C* could reduce the formation of that heterotrimer, increasing the amount of "free" $G\alpha_O$ in cells overexpressing $G\alpha_O2$. This would enhance its ability to antagonize $G\alpha_S$, ultimately leading to an increase in the penetrance of the crumpled wing phenotype.

Materials and Methods

Strains

w^{1118} (w[1118]; +; +) (Bloomington Fly Stock Center, Indiana University, Stock #3605); DJ858 (w[1118]; P{w[+mW.hs]=GawB}DJ858) (Seroude *et al.*, 2002); TM3,Sb balancer from 2475 (w*; $T(2;3)ap^{Xa}$, ap^{Xa}/CyO; TM3, Sb^1) (Bloomington Fly Stock Center, Indiana University, Stock #2475).

$G\alpha_O$ transgenic strains

P element-mediated transformation was performed according to standard protocol without removal of the chorion (Robertson *et al.*, 1988). 200 embryos were injected with the UAS-$G\alpha_O1$ construct, resulting in 11% fertile adults and a 3.5% transformation efficiency. 250 embryos were injected with the UAS-$G\alpha_O2$ construct, resulting in 17.2% fertile adults and a 3.6% transformation efficiency. $G\alpha_O1$ strains were named "VD2xy" and $G\alpha_O2$ strains were named "VD1xy"; "x" refers to the chromosomal location and "y" distinguishes between strains with the insertion on the same chromosome.

Crosses

All crosses were performed for 3-5 days at 25°C on food composed of 0.01% molasses, 8.2% cornmeal, 3.4% yeast extract, 0.94% agar, 0.18% benzoic acid, and 0.66% propionic acid. Progeny were scored 3-5 days after the first progeny emerged. *$G\alpha_O$ expression:* Virgin homozygous DJ858 females were crossed with males from each independent $G\alpha_O$ strain. *$G\alpha_O$-TM3 epistasis:* Virgin DJ858; TM3, Sb/+ females were crossed with homozygous males from $G\alpha_O2$ (strains VD131, VD133, and VD134) or $G\alpha_O1$ (strains VD231 and VD233).

References: de Sousa, S.M., *et al.*, 1989, J. Biol. Chem. 264: 18544-51; Di Gioacchino, V., 2014, Genetic evidence for differential activities of Goα isoforms in *Drosophila melanogaster*, MSc thesis, Queen's Unversity; Katanayeva, N., *et al.*, 2010, PLoS One 5: e12331; Kazemi-Esfarjani, P., and S. Benzer 2000, Science 287: 1837-40; Navon, S.E., and B.K. Fung 1987, J. Biol. Chem. 262: 15746-51; Robertson, H.M., *et al.*, 1988, Genetics 118: 461-70; Schmidt, C.J., *et al.*, 1989, Cell Regul. 1: 125-34; Seroude, L., 2002, Genesis 34: 34-8; Seroude, L., *et al.*, 2002, Aging Cell 1: 47-56; Slep, K.C., *et al.*, 2008, Proc. Natl. Acad. Sci. USA 105: 6243-8; St. Pierre, S.E., *et al.*, 2014, Nucleic Acids Res. 42(D1): 9; Thambi, N.C., *et al.*, 1989, J. Biol. Chem. 264: 18552-60; Yoon, J., *et al.*, 1989, J. Biol. Chem. 264: 18536-43.