Discussion

We have investigated any permanent impact on development stages of *Drosophila melanogaster* in this study. Thus F1 and F2 generations of *Drosophila melanogaster*, obtained from parents that were fed in bottles containing SDM with test compounds of Fumonisin B_1 chronically, were checked in terms of developmental stages of *Drosophila melanogaster*. But any effect has not been found. In addition to this, the number of F2 offspring was calculated in respect of gender and total number. A significant decrease was observed in all concentrations according to Control+DMSO (10%) (Table 1).

These results have concluded that Fumonisin B_1 has lasting effect on *Drosophila melanogaster*. There are very few reports available on the adverse effects of *Fusarium moniliforme* (Gelderblom *et al*., 1988). This fungus occurs worldwide on a variety of plant hosts (Nelson *et al*., 1991). Fumonisins are toxic metabolites of the fungus *Fusarium moniliforme*, which is a common contaminant of corn everywhere in the world. The fumonisins are carcinogenic in laboratory rats, and cause acute toxicity of domestic animals that mimics field cases of disease attributed to contamination of feed by *Fusarium moniliforme* (Norred, 1993). Fumonisin B_1 is the major fumonisin present both in cultures and in naturally contaminated samples (Nelson *et al*., 1991).

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Spatial and temporal variation in Drosophilidae (Diptera) abundance in three environments with different vegetal cover levels in a park in Porto Alegre, southern Brazil.

Garcia, Ana Cristina Lauer[1], Marco Silva Gottschalk[2], Martín Alejandro Montes[3], Victor Hugo Valiati[4], Claudia Rohde[1], Vera Lúcia da Silva Valente[5]. [1]Laboratório de Genética, Centro Acadêmico de Vitória, Universidade Federal de Pernambuco, Rua do Alto do Reservatório, s/n, 55608-680, Vitória de Santo Antão, PE, Brasil; [2]Laboratório de Ecologia de Insetos, Coordenação de Zoologia, Museu Paraense Emílio Goeldi, Avenida Magalhães Barata, 376, 66040-170, Belém, PA, Brasil; [3]Departamento de Biologia, Universidade Federal Rural de Pernambuco, Rua Dom Manuel de Medeiros s/n, 52171-900, Recife, PE, Brasil; [4]Laboratório de Biologia Molecular, PPG-Biologia, Universidade do Vale do Rio dos Sinos, Av. Unisinos, 950, 93022-000, São Leopoldo, RS, Brasil; [5]Departamento de Genética, Instituto de Biociências, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves, 9500, 91501-970, Porto Alegre, RS, Brasil; Corresponding author: alauergarcia@yahoo.com.br

Introduction

The Drosophilidae family encompasses 3,952 species distributed in 73 genera and two subfamilies (Steganinae and Drosophilinae). The subfamily Drosophilinae is the most diversified one, and includes 3,240 species distributed across 44 genera, of which *Drosophila* is the most speciose, with 1,159 species recorded (Bächli, 2009). Some of these species are endemic, while others are cosmopolitan and may often disperse in association with human activities (Tidon-Sklorz and Sene, 1999).

The *Drosophila* species are observed in essentially any environment, from the sea level to considerable altitudes, and in temperate as well as in equatorial zones (Throckmorton, 1975). However, Lachaise (1979) suggests that these species are subject to restrictions as regards the habitats they live in. Other authors have also stressed the trend exhibited by certain species towards occupying inner forests or else some sort of open environment (Dobzhansky and Pavan, 1950; Sene *et al.*, 1980). Studies that have evaluated drosophilid assemblages in forest fragments demonstrate the clear segregation between the faunistic composition of the inner forest and the disturbed areas. These same studies have revealed that species diversity increases gradually with forest fragment size (Martins, 1987, 1989). Climatic variables such as humidity, rainfall, temperature, and incidence of sunlight, among others, are determining factors in the occurrence of drosophilid species (Pavan, 1959). Similarly, biotic factors like the kind of vegetation that form natural gradients and changes associated to latitude, for example, are also important (Powell, 1997). Therefore, the composition and structure of a drosophilid assemblage depends on the habitat in which it was established. Studies on drosophilid assemblages aim at offering a simplified explanation of the complex systems and circumstances that are repeatedly observed in different habitats. The recognition of patterns at these organizational levels affords to propose hypotheses about the ecology and evolution of the groups studied (Begon *et al.,* 1990). Due to increased global habitat destruction, studies on the diversity of insects in various environments are highly important regarding the comprehension of biological assemblages and the devising of conservation strategies (Purvis and Hector, 2000).

The present study provides information on the spatial and temporal structure of the Drosophilidae family in three environments (open field, forest edge and inner forest) along the four seasons during one year in Gabriel Knijnik Park, in the city of Porto Alegre, southern Brazil.

Materials and Methods

Adult drosophilids were collected in 2004, in Gabriel Knijnik Park (30º06’12.6”S, 51º12’10.5”W). The park covers an area of 119,545 m² and is located in the city of Porto Alegre, southern Brazil (Figure 1). Collections were made in the four seasons of the year: summer (February), autumn (April), winter (July) and spring (October). Three environments in the park were evaluated and classified according to the vegetal cover: (i) field, (ii) forest edge, and (iii) inner forest (Figure 2). The collection sites in the inner forest were located at least 200 m away from the forest edge.

To attract Drosophilidae flies 5 kg of banana and 5 kg of orange placed on the ground and covered with yeast were used. Baits were distributed as identical amounts in the three studied environments. After three days, the insects were captured with entomological nets and taken to the laboratory where they were identified by their external morphology. Sibling species identification was carried out by dissection and analysis of male genitalia. The species belonging to the *willistoni* subgroup of *Drosophila* (*D. willistoni* and *D. paulistorum*) were identified by electrophoresis of the Acid Phosphatase-1 enzyme (Acph-1), according to Garcia *et al.* (2006).

Drosophilid populations were compared using the absolute numbers and frequencies of the species recorded. The χ^2 test was used to assess the variations in these species' frequencies across the different environments and seasons of the year.

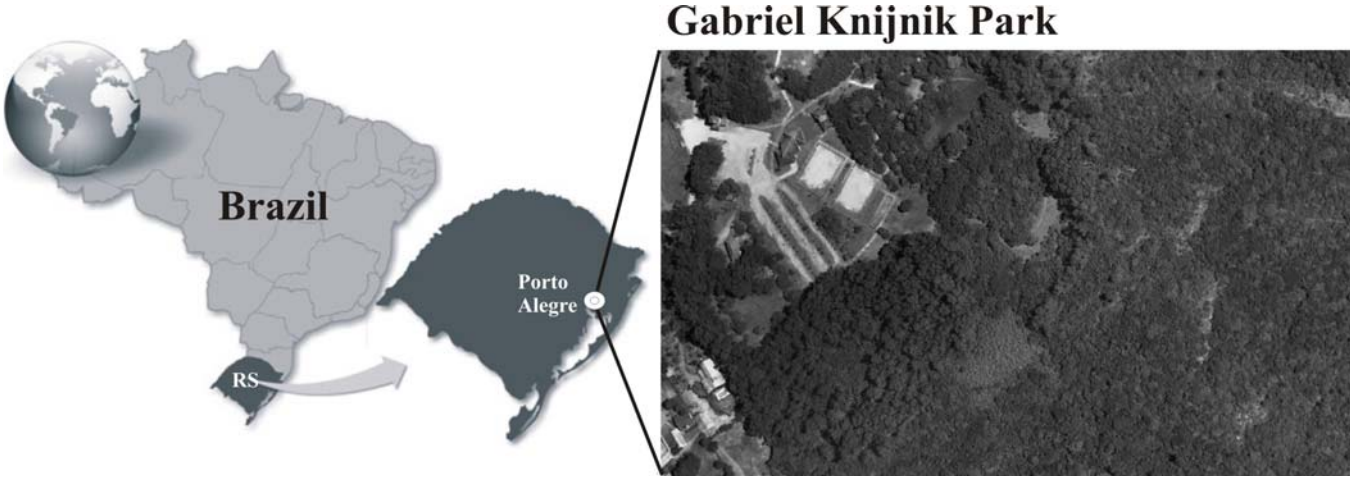

Figure 1. Map of Brazil showing the state of Rio Grande do Sul (RS), where the city of Porto Alegre is located (white circle). The zoomed out image shows an aerial photograph of Gabriel Knijnik Park.

Figure 2. Areas sampled in Gabriel Knijnik Park, Porto Alegre, Brazil. A = Field; B = Forest edge; C = Inner forest.

Results and Discussion

The collections conducted throughout 2004, in the four seasons, in the three environments of Gabriel Knijnik Park afforded to gather 1,377 individuals that belonged to 25 species of the Drosophilidae family. From these, five species were exotic (*Zaprionus indianus*, *Drosophila simulans*, *D. melanogaster*, *D. ananassae*, and *D. immigrans*) (Table 1).

Table 1. Absolute number of drosophilids collected in the four seasons in the year 2004, in the three environments investigated in Gabriel Knijnik Park, Porto Alegre, Brazil. F = Field; E = Forest edge; I= Inner forest.

Species	Author, year	Seasons												TOTAL (%)
		Summer			Autumn			Winter			Spring			
		F	E	I	F	E	I	F	E	I	F	E	I	
Drosophila willistoni	Sturtevant, 1916	12	154	62	37	40	143	4	2	6	4	4	5	473 (34.35)
D. simulans†	Sturtevant, 1919	165	48	2	47	18	33	28	4	0	4	1	0	350 (25.42)
D. mediopunctata	Dobzhansky and Pavan, 1943	0	0	1	0	0	1	11	33	187	12	25	25	295 (21.42)
Zaprionus indianus†	Gupta, 1970	16	7	0	51	18	3	0	0	0	0	0	0	95 (6.90)
D. mediosignata	Dobzhansky and Pavan, 1943	0	0	0	0	0	0	1	2	17	0	0	0	20 (1.45)
D. griseolineata	Duda, 1927	0	4	1	0	0	6	0	1	3	3	0	2	20 (1.45)
D. maculifrons	Duda, 1927	0	0	0	0	0	0	2	2	11	0	2	0	17 (1.23)
D. capricorni	Dobzhansky and Pavan, 1943	0	10	1	0	0	0	0	0	4	0	0	0	15 (1.09)
D. polymorpha	Dobzhansky and Pavan, 1943	0	5	0	0	1	0	4	0	3	0	0	0	13 (0.94)
D. melanogaster†	Meigen, 1830	0	12	0	0	0	0	0	0	0	0	0	0	12 (0.87)
D. nappae	Vilela *et al*., 2004	0	0	0	0	0	0	0	0	12	0	0	0	12 (0.87)
D. paulistorum	Dobzhansky and Pavan, 1949	0	3	2	0	0	4	0	0	0	2	0	0	11 (0.80)
D. nebulosa	Sturtevant, 1916	0	2	0	4	3	1	0	0	0	0	0	0	10 (0.73)
D. bocainensis	Pavan and Da Cunha, 1947	0	0	0	0	0	0	0	1	2	0	3	1	7 (0.51)
D. bandeirantorum	Dobzhansky and Pavan, 1943	0	0	0	0	0	0	0	0	2	0	0	4	6 (0.44)
D. immigrans†	Sturtevant, 1921	0	1	0	0	0	0	0	1	0	3	0	1	6 (0.44)
D. mercatorum	Patterson and Wheller, 1942	0	0	0	1	0	0	2	0	1	0	0	0	4 (0,29)
D. sturtevanti	Duda, 1927	0	1	1	0	0	1	0	0	0	0	0	0	3 (0.22)
D. angustibucca	Duda, 1925	0	0	0	0	0	0	0	0	0	1	0	1	2 (0.14)
D. neocardini	Streisinger, 1946	0	1	0	0	0	0	0	0	0	0	0	0	1 (*)
D. pallidipennis	Dobzhansky and Pavan, 1943	0	0	0	0	0	0	1	0	0	0	0	0	1 (*)
D. ornatifrons	Duda, 1927	0	0	0	0	0	0	1	0	0	0	0	0	1 (*)
D. annulimana	Duda, 1927	0	0	0	0	0	0	0	0	1	0	0	0	1 (*)
D. ananassae†	Doleschall, 1858	0	0	0	0	0	0	0	0	1	0	0	0	1 (*)
D. parabocainensis	Carson, 1954	0	0	0	0	0	0	0	0	0	0	0	1	1 (*)
TOTAL (%)		193 (14.02)	248 (18.01)	70 (5.08)	140 (10.17)	80 (5.81)	192 (13.94)	54 (3.92)	46 (3.34)	250 (18.16)	29 (2.11)	35 (2.54)	40 (2.91)	1377

(*) Frequency under 0.1%. *†* Exotic species.

The highest absolute number of individuals was collected in summer (511 specimens), after which came autumn (412), winter (350) and spring (104). The highest species diversity was observed in winter (17 species), followed by summer (13), spring (11), and autumn (10) (Table 1).

When the total number of individuals collected throughout the year is considered, the most abundant species were *D. willistoni* (34.35%), *D. simulans* (25.42%), *D. mediopunctata* (21.42%), and *Z. indianus* (6.90%). Species representativeness varied across the sampling periods, for the species listed above. *Drosophila willistoni*, *D. simulans,* and *Z. indianus* occurred at significantly higher frequencies in summer ($\chi^2 = 378.73$; df = 3; $P < 0.0001$) and autumn ($\chi^2 = 255.43$; df = 3; $P < 0.0001$). In opposition, *D. mediopunctata* was significantly more frequent in winter ($\chi^2 = 518.15$; df = 3; $P < 0.0001$) and spring ($\chi^2 = 121.90$; df = 3; $P < 0.0001$) (Figure 3).

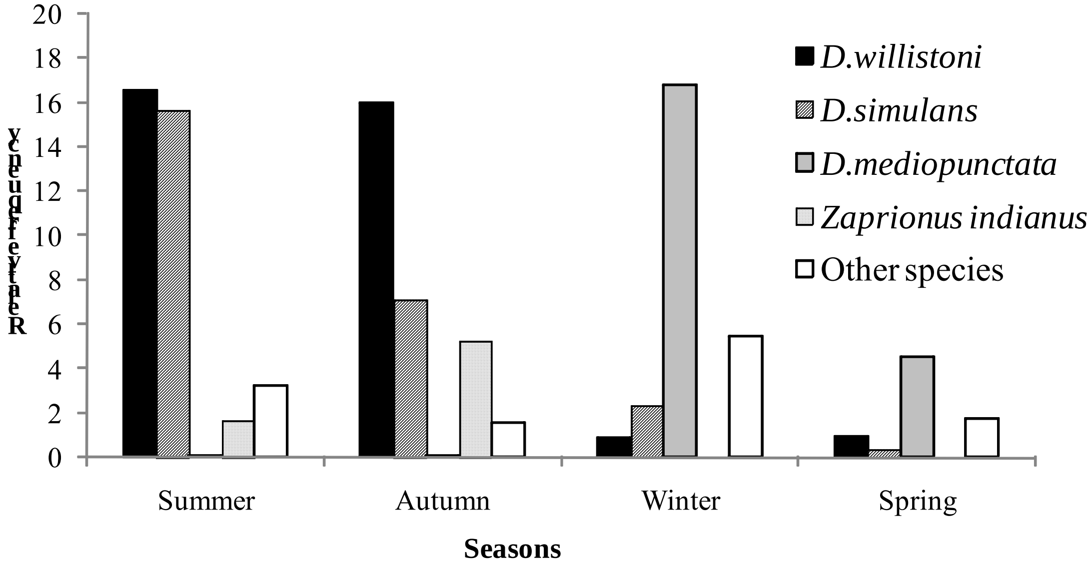

Figure 3. Relative frequency of the most abundant drosophilids collected along the four seasons of the year 2004 in Gabriel Knijnik Park, Porto Alegre, Brazil.

Several studies have been carried out in natural environments in southern Brazil (Frank and Valente, 1985; Valente and Araújo, 1991; Saavedra *et al.*, 1995; Döge, 2006), all of which pointed to peaks in *D. willistoni* abundances in autumns and summers, as observed in the present study. Our results for *D. mediopunctata* likewise agree with the literature data, which demonstrate that this species is often observed in winter (Sene *et al.*, 1980; Saavedra *et al.*, 1995). This species' maturity span is quite long, with the first eggs being laid on the seventh day after eclosion. Also, *D. mediopunctata* fecundity is low and longevity is high. Temperature increases have been pointed to affect positively the population size of *D. simulans* (Döge, 2006), an exotic species commonly observed in forests across Brazil and especially in disturbed environments (Saavedra *et al.*, 1995). This is possibly due to the fact that this species' growth speed rises with temperature, reaching its optimum at 29ºC (Cohet *et al.*, 1979). Similarly, in the present study *D. simulans* was at its most frequent in summer, when temperatures are higher.

According to the theoretical model proposed by Sevenster and Van Alphen (1993), short life cycle species prevail when resources abound, since these species consume these supplies more quickly, excluding other species by competition. This could be seen as an explanation for the overwhelming dominance of *D. willistoni* in summer and autumn. On the other hand, in a scenario of paucity of resources species with longer life cycles are at an advantage, since they have higher longevity values and lower metabolic rates and, therefore, exhibit greater probabilities of success in finding an oviposition site. This explains the expansion and dominance of *D. capricorni* and of the species of the *tripunctata* group in winter.

As for the seasonal oscillations in *Z. indianus* frequencies, an exotic species that invaded the Brazilian territory in the 1990's (Vilela, 1999), several studies have demonstrated the species' limitation to colder temperatures. Under lower temperatures, *Z. indianus* abundances dwindle and may even disappear completely from one environment (Gottschalk *et al.*, 2007; Silva *et al.*, 2005a,b; Garcia *et al.*, 2008). In a recent study also carried out in the city of Porto Alegre, Garcia *et al.* (2008) have observed that very low temperatures constitute a limiting factor concerning *Z. indianus* population size.

When the different environments sampled in Gabriel Knijnik Park are considered, the highest number of species was recorded for the inner forest (21), followed by the forest edge (16) and field (15). In all sampling periods specified in the present study, the highest number of individuals was likewise collected in the inner forest (552). Field and forest edge came next, producing 416 and 409 specimens, respectively. It was also observed that the most abundant exotic species, *Z. indianus* and *D. simulans*, exhibited a significant preference for the field areas ($\chi^2 = 302.64$; df = 3; $P < 0.0001$), while *D. mediopunctata* and *D. willistoni*, the most common native species in all sampling periods, were significantly the prevailing species in occupying the inner forest ($\chi^2 = 332.73$; df = 3; $P < 0.0001$). In the forest edge, the representativeness of *D. willistoni* was significantly higher ($\chi^2 = 200.01$; df = 3; $P < 0.0001$) (Figure 4).

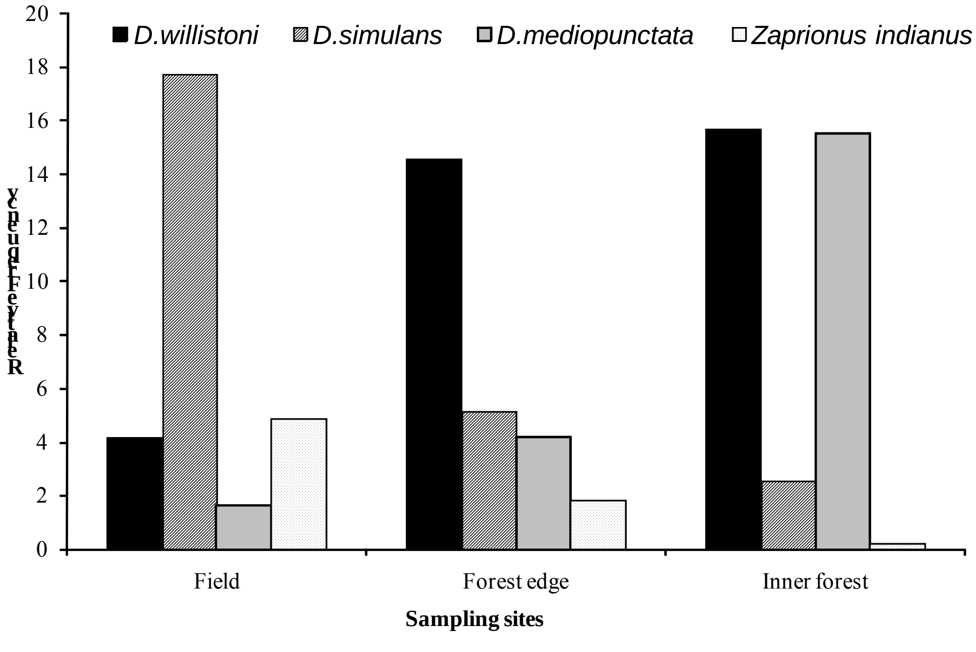

Figure 4. Relative frequency of the most abundant drosophilids collected in the three environments investigated in Gabriel Knijnik Park, Porto Alegre, Brazil.

Drosophila willistoni is the dominant species in tropical and subtropical forest areas in the Neotropical region (Dobzhansky and Pavan, 1950; Erhman and Powell, 1982; De Toni and Hofmann, 1995; Saavedra *et al.*, 1995; Döge, 2006). This species occurs in higher numbers in more shady and humid areas (Martins, 1987), which has also been observed in the present study, manifested in the preference for the forest edge and inner forest. The predilection for forest environments exhibited by *D. mediopunctata* was verified by Mata *et al.* (2008). *Zaprionus indianus* has been consistently observed in open environments (Tidon *et al.*, 2003; Mata *et al.*, 2008) and urbanized (Gottschalk *et al.*, 2007), though it is rare in forests (Döge, 2006). *Drosophila simulans*, like *Z. indianus*, has higher relative frequency in open or dry environments as the Cerrado and the Caatinga, and thus it is considered adapted to arid conditions (Sene *et al.*, 1980; Vilela *et al.*, 1983; Tidon *et al.*, 2003; Tidon *et al.*, 2005).

As regards the species recorded at lower frequencies in the present study, as a rule some were rarely observed, considering the four seasons and the three environments altogether. Yet, they were more consistently represented when their populations were assessed considering a specific season of the year and/or one of the environments studied. For instance, in the forest edge *D. melanogaster* was recorded only in summer and was the third most common species in this environment and in this season, representing 4.83% of the drosphilids collected (Table 1). The species is appointed as characteristic of urban environments (Mata *et al.*, 2008), and some authors have revealed that it is most abundant in summer (Parsons and Stanley, 1981). *Drosophila nappae* occurred only in the inner forest, in winter, when it was recorded at a frequency similar to 5%, being the third more frequent species in that environment and in that season. In a study conducted in the state of Santa Catarina, southern Brazil, Döge (2006) also observed the greater representativeness of the species in winter, in an inner forest environment.

Drosophila capricorni exhibited a higher occurrence in summer (2.15%) and, in this season, the species was detected in the forest edge as well as in the inner forest. Although the species has been associated to cold climates (Dobzhansky and Pavan, 1950), only four specimens were collected in winter in the present study, accounting for 1.14% of the individuals collected in this season. Also, these four *D. capricorni* specimens collected in winter were recorded only in the inner forest (Table 1).

The frequencies of the most abundant species recorded in the present study fluctuated consistently between seasons. Similarly, the structures of drosophild assemblages recorded in the inner forest, forest edge, and field were observed to be markedly different from one another. These results underline the importance of understanding the patterns of temporal and spatial population oscillations as a means to assist in the development of conservation strategies. Also, these findings may be used as a tool in the implementation of conservation areas and the design of parks with vegetal covers that are large enough to work as ecological sanctuaries to several species that, as a rule, tend to decrease in frequency due to the urbanization and the arrival of colonizing species.

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Expression of Gal4 alone alters DNA replication and causes cell death in ovarian follicle cells.

McConnell, Kristopher H., and Brian R. Calvi. Department of Biology, Indiana University, Bloomington, Indiana. Corresponding author: Brian R. Calvi: bcalvi@indiana.edu

Drosophila melanogaster is a powerful model organism for biological research in large part because of the many versatile genetic tools available to the fly geneticist. One of the most powerful tools is the Gal4-UAS system, which uses the yeast transcription factor Gal4 to drive expression of transgenic constructs within the developing organism (Brand and Perrimon 1993). This system has been expanded and modified to allow exquisite spatial and temporal control of expression (McGuire et al 2004). The importance of the Gal4-UAS system cannot be overstated. However, the utility of the system depends on the expression of Gal4 alone having no confounding effects on the cellular or developmental process being studied.

We have found that expression of Gal4 in ovarian follicle cells can result in disrupted developmental gene amplification, cell death, and altered egg chamber morphology. During oogenesis, the oocyte is surrounded by a layer of epithelial cells known as follicle cells which secrete proteins essential for chorion (eggshell) synthesis (see Calvi, 2006, for review). To support rapid eggshell synthesis, the DNA copy number of chorion and other genes are amplified by repeated rounds of DNA re-replication, a process known as developmental gene amplification. At Stage 10B of oogenesis, genomic DNA replication shuts down, and amplification begins at six discrete sites within the genome (Claycomb *et al.*, 2004). This amplification can be seen as nuclear foci of BrdU incorporation from stage 10B to 13 (Figure 1A, Calvi *et al.*, 1998). Surprisingly, we found that induction of an *Hsp70:Gal4* on the 3^{rd} chromosome (Brand and Perrimon, 1993) alters BrdU