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Cellular aspects of gonadal atrophy in *Drosophila* P-M hybrid dysgenesis

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ABSTRACT

Gonadal atrophy is the most typical and dramatic manifestation of intraspecific hybrid dysgenesis syndrome leading to sterility in *Drosophila melanogaster* dysgenic progeny. The P-M system of hybrid dysgenesis is primarily associated with germ cell degeneration during the early stages of *Drosophila* embryonic development at elevated temperatures. In the present study, we have defined the phase of germ cell death as beginning at the end of embryogenesis immediately following gonad formation. However, the temperature-dependent screening of germ cell developmental patterns in the dysgenic background showed that early germ cells are susceptible to the hybrid dysgenesis at any *Drosophila* life-cycle stage, including in the imago. Electron microscopy of germ cells after dysgenesis induction revealed significant changes in subcellular structure, especially mitochondria, prior to cellular breakdown. The mitochondrial pathology can promote the activation of cell death pathways in dysgenic germ cells, which leads to gonadal atrophy.

1. Introduction

Hybrid dysgenesis (HD) is characterized as a *Drosophila* sterility syndrome that occurs in the F1 offspring of some intraspecific crosses. P-M hybrid dysgenesis (P-M HD) results from an interaction between the paternal (P) genome and the maternal (M) cytoplasm and is induced when M-females are crossed to P-males (Kidwell et al., 1977). The reciprocal crosses are nondysgenic. The PM HD phenotype is susceptible to developmental temperature and is strongly displayed when dysgenic flies are raised at restrictive temperatures of 25–29 °C. Lowering the temperature to 20 °C usually completely prevents dysgenic symptoms (Engels, 1979; Kidwell and Novy, 1979). Dysgenic gonads contain an extremely reduced number of germ cells; however, a normal somatic background is maintained (Kidwell and Novy, 1979; Engels and Preston, 1979; Bhat and Schedl, 1997).

According to the dominant hypothesis, P-strain genomes contain *P* elements that are repressed via PIWI-interacting microRNAs (piRNA) maternally stored in the eggs. M-strains do not carry P-elements as well as transposon silencing machinery. Dysgenic offspring inherit the M– cytotype, which allows the P-element to become active. Uncontrolled P-element mobilization in the germline causes a destruction of genome integrity leading to gonadal atrophy and sterility. This hypothesis explains the HD effect but is contrary to some experimental data (Malone et al., 2015). The problem appears to be more complex, and severe developmental consequences caused by HD cannot simply be explained by transposon activation alone (Malone et al., 2015).

For many decades, HD has been studied as a model for the analysis of P-element transposition, its structure and regulatory mechanisms (especially silencing machinery). However, the phenomenon of massive germline degradation deserves attention because the mechanism leading to germ cells absence is unknown. Nevertheless, different causes are assumed including loss of specification, abnormal cell division, cell cycle arrest due to multiple DNA breaks, and others (Engels, 1996; Bhat and Schedl, 1997; Majumdar and Rio, 2015). However, to understand the underlying mechanisms of this phenomenon it is necessary to determine what cellular event or process is the primary target and which developmental stage is the most sensitive. Therefore, to understand germ cell fates in the dysgenic background we monitored their development cytologically during the fly life cycle under various temperature conditions.

In this study, we focused on germ cell fates in the PM HD system. We showed that germ cells of dysgenic hybrids participate in embryonic gonad formation; however, most subsequently die amidst the survival of the somatic cells. Nevertheless, the phase of germ cell death is not exclusively embryonically determined and can occur during any stage of the *Drosophila* life cycle by altering the temperature conditions. In particular, we show that if the dysgenic flies are raised at 20 °C and then moved to 29 °C, their germ cells begin to lyse quickly en masse and then die (during 3–5 days). However, pathological changes in the cytoplasm were observed most notably with the appearance of a large number of damaged mitochondria prior to germ cell death. We propose that dramatic alteration in mitochondria can be involved in the cell death process and contribute to germline atrophy.

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2. Materials and methods

2.1. Drosophila *culture and strains*

We used *D. melanogaster* strains: *Canton S* obtained from I. Zakharov, Novosibirsk, Russia (maintained since 1968); *Harwich-w* obtained in 1996 from L. Kaidanov, Saint-Petersburg, Russia, and saved in the laboratory of Prof. I. Zakharov from Novosibirsk.

Control and dysgenic crosses were set up at the permissive temperature of 20 °C and at the restrictive temperatures of 25 and 29 °C.

2.2. *Electron and fluorescence microscopy*

Experimental procedures for electron and fluorescence microscopy were performed as described previously (Pertceva et al., 2010). Antibodies used were monoclonal anti-VASA (SC 30210, Santa Cruz Biotechnology; 1:300), anti-Fasciclin III (7G10, DSHB Hybridoma Product; 1:80), polyclonal guinea pig anti-Traffic Jam [kindly provided by Prof. D. Godt (Li et al., 2003), 1:400], Alexa-488-conjugated anti-guinea pig (A-11073) and anti-mouse IgG (A-11001), and Alexa-568-conjugated anti-rabbit IgG(A-11011) (Molecular Probes/*Invitrogen;* 1:400). We used *ProLong Gold* anti-fade reagent with DAPI staining (Molecular Probes/*Invitrogen).*

For embryonic antibody staining, embryos were removed from agar plates with a paintbrush and dechorionated in 50% bleach for 5 min, followed by a cold water rinse to remove the bleach. Embryos were then fixed in 3.7% formaldehyde and heptane, devitellinised with methanol/heptane and stained with antibodies using standard protocols (Patel, 1994).

Lysosome staining was carried out in 1 μM LysoTracker red DND-99 (Invitrogen, Molecular Probes, Basel, Switzerland) according to a previously described procedure (Dorogova et al., 2014). For ROS detection, non-fixed gonads were incubated in 0,05 mM CellROX® Deep Red Reagent (C 10422, Life Technologies) in Hank's solution at +37 °C during 30 min. Then gonads were fixed and stained by DAPI as described above.

Images were obtained using an AxioImager Z1 microscope with ApoTome attachment (Zeiss), AxioCam MR and AxioVision software (Zeiss, Germany).

2.3. *Quantification and statistical analysis*

After immunostaining, the number of germ cells and germ cell cysts was determined by counting VASA-positive cells from optical serial sections. Average and standard derivation are calculated.

Quantification of relative ROS fluorescence intensity. Mean intensities of individual germarium were measured using an AxioVision software (Zeiss). Fluorescence intensities are normalized to the average intensity of unstained egg chambers from the same slide. The values from 30 germaria were averaged.

3. Results and discussion

P-M HD results in full female sterility and a half-sterility of males raised at 29 °C. Dysgenic flies raised at this temperature had reduced gonads with an absence or significantly reduced number of germline cells. However, infertility can be prevented partially or completely when the developmental temperature is lowered to 25–20 °C (Engels and Preston, 1979; Kidwell and Novy, 1979). We used the temperature effect on the fate of germ cells to identify what stage (or event) of their development is targeted in dysgenesis.

To generate offspring with dysgenesis syndrome we crossed *CantonS* females (M strain) to *Harwich* males (P strain). The first control was the *CantonS* strain because these females are a source of germ plasm, and the second control were the offspring from a reciprocal cross because they are genetically identical to dysgenic F1. However, the data presented result from only one control because their comparison did not show significant differences.

3.1. *Phenotypic manifestation of P-M hybrid dysgenesis*

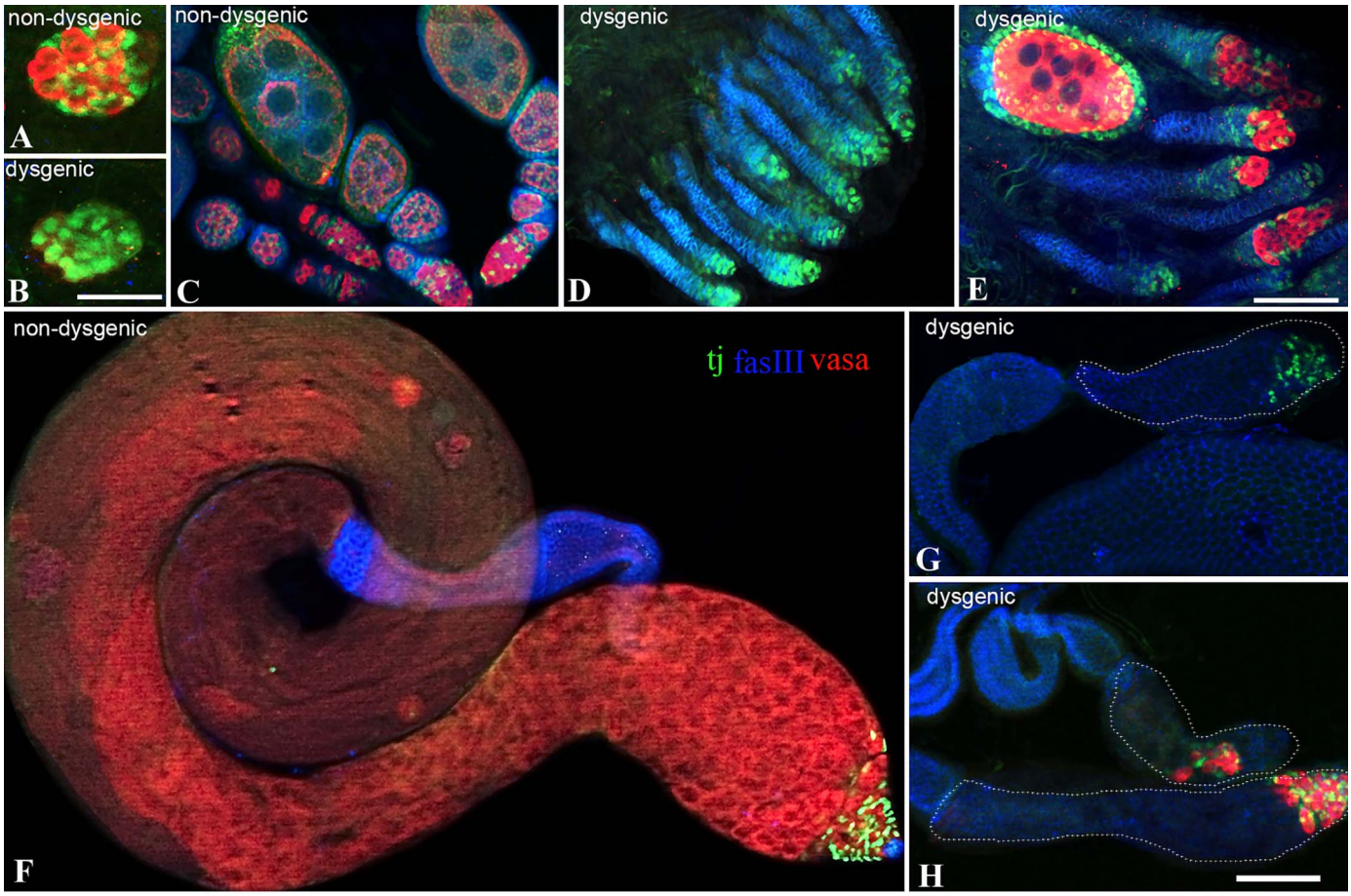
We have conducted a detailed cytological analysis of the cell organization and morphology in dysgenic gonads subjected to alterations in the incubation temperature from restrictive to permissive (temperature shift) at different stages of the *Drosophila* life cycle. Cells were visualized with antibodies against the VASA protein to mark the germline and antibodies against the Traffic Jam and Fasciclin III proteins to mark soma.

According to literary data (Schaefer et al., 1979; Engels and Preston, 1979; Bhat and Schedl, 1997) and our observations, HD generates two main phenotypes associated with the reduction of germ cells. The first dysgenic phenotype (phenotype 1) represents a complete loss of germ cells and the formation of rudimentary gonads consisting only of somatic cells. The second phenotype (phenotype 2) is a partial germ cell deficiency and, as a consequence, the formation of the gonads with single egg chambers in females and single cysts in males.

Previous studies using particular temperature shift experiments revealed that phenotype 1 appears maximally (~100%) when dysgenic progeny are raised at 29 °C from mid-embryogenesis to the early larval period (Engels and Preston, 1979; Bhat and Schedl, 1997). To define when the germ cells are lost, we dissected gonads of dysgenic embryos incubated at 29 °C. Detailed cytological analysis showed that germ cells were clearly visible until stages 15–16. Despite some migratory defects described previously (Ignatenko et al., 2015), these cells reached the somatic gonadal precursors in the mesoderm and assembled with them into two compact groups to form the embryonic gonads. However, the germ cells subsequently degenerated, and the gonads became bands of somatic cells (Fig. 1A and B). The presence of Traffic Jam specifically in somatic gonad precursors revealed that germ cell loss occurred in approximately 70% of embryos that were stage 15 or older (n=200). Almost all of the gonads of the adult flies (99% female and 60% male) (n=100) that had developed from embryos incubated at 29 °C were devoid of germ cells but contained cells of somatic origin (Fig. 1D and G). In this case, germ cell loss was total and recovery of these cells did not occur during development even after lowering the temperature to permissive levels after the embryonic stages. Thus, maintaining dysgenic offspring at 29 °C only during the embryonic stage is sufficient to generate phenotype 1 (fully rudimentary gonads).

Phenotype 2, or partially rudimentary gonads, appears when the developmental temperature is lowered to 25 °C. This condition allows the survival of some germ cells in the dysgenic background. The dysgenic flies must be maintained at 25 °C throughout and after embryogenesis to obtain an absence of germ cells. The strongest phenotypes and significant germline reduction in both sexes are observed when hybrid progeny are grown at 25 °C throughout their life cycle (Fig. 1E, H, I). Phenotype 2 is extremely pleiotropic. For example, such gonads contain ovarioles lacking germ cells, ovarioles with late-stage egg chambers (1–3) and an empty germarium, or, conversely, only early-stage cells may be represented (Fig. 1E). The phenotype 2 manifestations suggest that HD may be inducible not only during the embryonic period. The presence of ovarioles comprised of egg chambers and empty germaria suggests that early germ cells (including stem cells) degraded after they had given rise to the subsequent stages of oogenesis, but not until pupal stages when differentiation begins. An increase in the number of fully reduced ovarioles in response to an extended restrictive period also indicates a dysgenic effect following embryogenesis.

Based on this information, we have concluded that germ cell viability is not determined during specific events of embryogenesis (specification, proliferation, or migration), but immediately as the gonads form. Mitigation of dysgenic conditions (25 °C) allows some



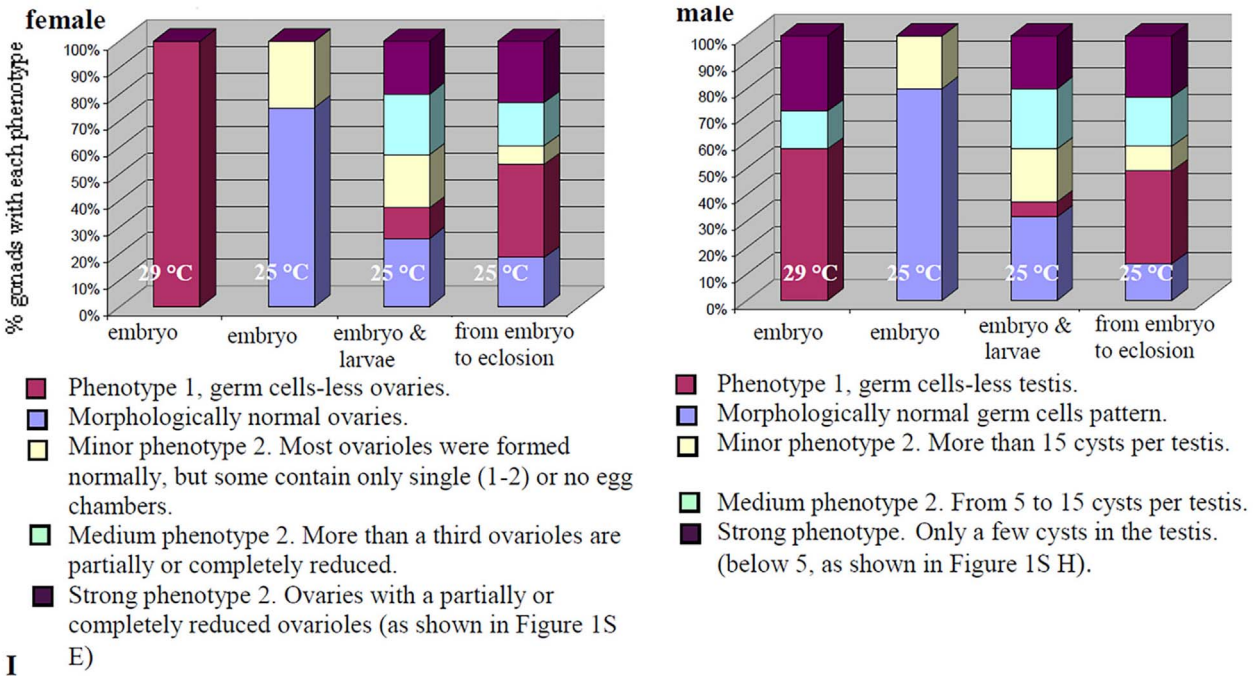


Fig. 1. *Drosophila* gonad formation under conditions inducing hybrid dysgenesis. A,B. Gonads of control non-dysgenic (*Harwich/CantonS*) (A) and dysgenic (*CantonS/Harwich*) crosses (B). Stage 15–16 embryos raised at 29 °C. A. In the control, the embryonic gonad includes interacting germ and somatic cells. B. The dysgenic embryonic gonads are rudimentary and only contain somatic cells. C-E. Adult 1-day-old female ovaries in the control and dysgenic flies at the same magnification. C. The cellular structure of the gonads appears uniform in the control, regardless of temperature; gonads are filled with germ cells in combination with somatic cells. D. Ovaries of dysgenic females grown at 29 °C. Germ cells are absent, however cells and structures of somatic origin are retained. E. Ovaries of dysgenic females grown at 25 °C. There is no oogenesis and egg chamber formation in most ovarioles. Germ cells are present mainly in the early regions of the germarium. F-H. Cell structure of control (F) and dysgenic testes at 29 °C (G) and 25 °C (H). F. A control testis filled with germ cells, which are grouped in cysts corresponding to the different stages of spermatogenesis, and this phenotype is temperature independent. G. Dysgenic testes exhibited a significant reduction in size and absence of germ cells. H. Testis of a dysgenic male raised at 25 °C. It is significantly smaller in size and contains only a few germ cells. Germ cells are visualized by anti-Vasa (red), somatic cells – anti-FasIII (blue) and anti-TJ (green). Scale bar: 5 μm (A,B); 20 μm (C-H). Anterior of the right. I. Phenotypic variability of germ cell reduction in dysgenic gonad depending on period of incubation at restrictive temperature. The experiment was repeated five-six times. A minimum of 500 embryo gonad, 100 ovaries and 100 testes were analyzed as in control and dysgenic crosses.

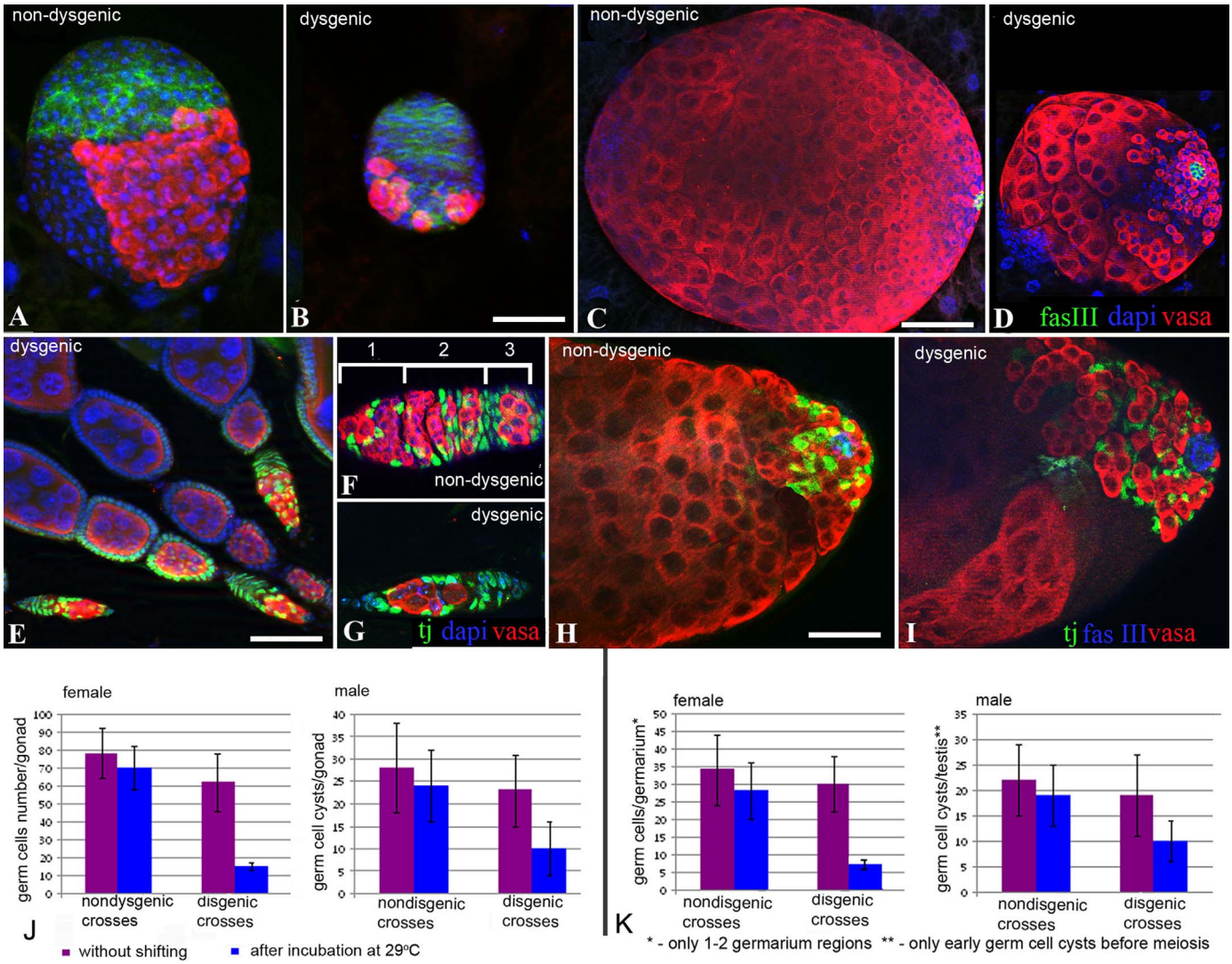


Fig. 2. Dysgenesis-induced germ cell degeneration under developmental temperature variation from 20 to 29 °C. A–D. Larval gonads after incubation at 29 °C for 3 days. A. Control larval ovaries have a normal germ cell pattern B. Dysgenic ovaries are much smaller and contain very few germ cells. C,D. Larval dysgenic testes (D) are reduced in both size and number of germ cells compared to the control (C). E-I. Imago gonad after incubation of newly hatched flies at 29 °C for 3 days. E. Dysgenic imago ovarioles contain reduced germaria but normal egg chambers at later stages. F,G. Comparison of germaria in control (F) and dysgenic (G) ovaries. Dysgenic germaria contain significantly fewer germ cells than in the control and they are not differentiated in the regions of 1-2A-2B-3. H. In the control testis, the anterior end is completely filled with early germ cells. I. In dysgenic testes, there are clearly identified VASA-negative areas lacking germ cells. J,K. Quantification of the germ cell numbers in the female gonads and the germ cells cysts in the male gonads after incubation at 29 °C for larvae stage (J) and for 3 days after hatching (K). Germ cells are visualized by anti-Vasa (red), somatic cells – anti-FasIII (green), DNA - DAPI (blue). Non-dysgenic – *Harwich*/*CantonS*, dysgenic - *CantonS*/*Harwich*. Scale bar: 5 μm (A, B); 10 μm (C-D); 20 μm (F-I). We repeated this analysis at least five times. Not less than 100 gonads were examined for each developmental stage, as in control and in hybrid fly.

germ cells to be rescued in embryogenesis but they remain susceptible beyond this stage.

3.2. Hybrid dysgenesis affects early germ cells, regardless of the ontogenetic stages

Since germ cells are eliminated in already formed gonads, we assumed that this effect can be promoted at any stage of the fly life cycle. For providing HD conditions, dysgenic offspring of different developmental stages (larva, pupa or imago) were placed from 20 to 29 °C. Maintenance of dysgenic offspring at 20 °C beforehand avoids the effects of hybrid dysgenesis and provides normal gonad development.

Altering the developmental temperature from 20 to 29 °C for larvae and pupae induced germ cell degradation. Germ cell reduction was most apparent on the 3rd day of incubation at the restrictive temperature (Fig. 2A–D, J). Partial loss of germ cells were observed in all gonads, however, only 3% of the females and 1% of the males demonstrated complete atrophy of germline tissue (n=100).

In 1-day-old flies reared at 20 °C followed by the restrictive temperature for 3 days, the dysgenic gonads showed general germ cell depletion in the germarium of the ovaries and apical end of the testes where early-stage cysts are located (Fig. 2E–I, K). Most later-stage germ cells were resistant to the HD effect, and they developed normally to produce eggs and sperm. Females maintained at 29 °C for one week had significantly reduced reproductive systems: completely atrophied germaria and also significantly depleted vitellaria resulting from the inability of the germaria to produce new egg chambers. Males that developed in the same conditions also demonstrated a depletion of germ cell cysts especially in the anterior region of the testes. All of the analyzed dysgenic gonads (n~200) showed significant and obvious early-stage germ cell loss.

Thus, the temperature increase is a trigger of hybrid dysgenesis phenotype regardless of the stage of the *Drosophila* life cycle, and germ cells in the early stages of their development are the most sensitive. Embryonic gonads consist exclusively of early-stage germ cells and consequently undergo rapid atrophy.

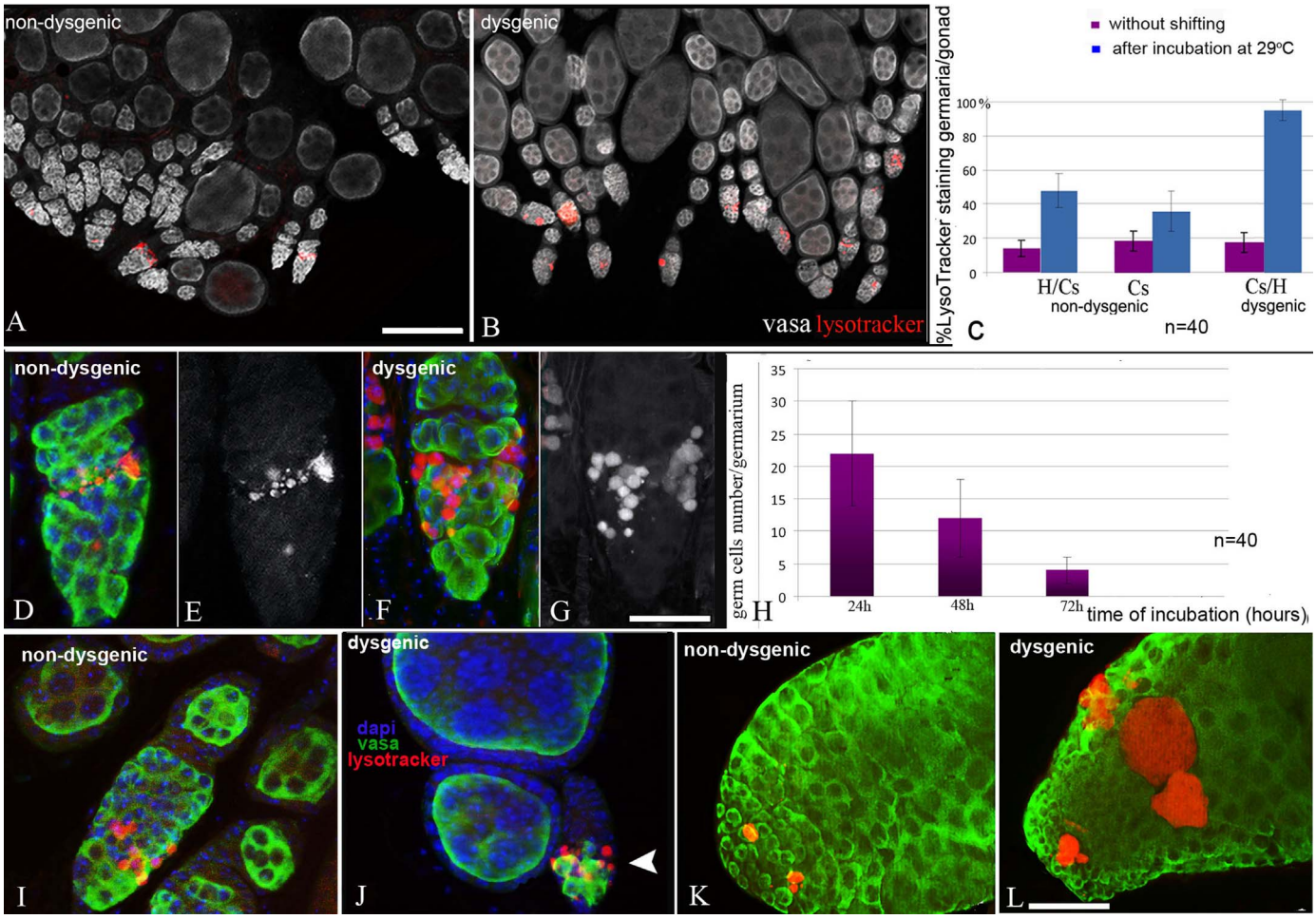


Fig. 3. Lysis of germ cells, caused by hybrid dysgenesis. A,B. The ovaries of 1-day-old females after a 3-h incubation at 29 °C. LysoTracker staining is observed in almost all germaria in dysgenic ovaries (B) in contrast to the control (A).C. Percentage of the LysoTracker staining germaria in control and dysgenic ovaries. D-G. LysoTracker-stained areas in control and dysgenic germaria after incubation at 29 °C for3 hours. The LysoTracker-labelled area is wider in dysgenic progeny (F, G) than in controls (D,E) and extends to different regions of germarium. H. Germ cells reduction in dysgenic germarium in depending on time of incubation of adult female flies at 29 °C. I,J. germ cell lysis after a 3-day incubation at 29 °C. I. In the control, LysoTracker stains only a local area of the germarium. J. In dysgenic ovaries, germarial regions 2B-3 are absent, and the remaining germ cells are covered by lysis. K,L. Testes of control (K) and dysgenic (L) males after a 3-day incubation at 29 °C. There is massive lysis in dysgenic testes. Germ cells are visualized by anti-Vasa (green), DNA - DAPI (blue), lysosomes – LysoTracker (red). Non-dysgenic - *Harwich/CantonS*, dysgenic - *CantonS/Harwich*. Scale bars: 50 μm (A,B); 15 μm (D-E); 10 μm (F-G). Data were collected from at least three different experiments, and a minimum of 100 control and 100 dysgenic ovaries and testes were analyzed.

3.3. Hybrid dysgenesis induces massive germ cell lysis

The temperature-dependent screening of gonadal phenotypes showed that HD can cause significant early germ cell loss at any stage of fly development. To determine whether germ cells die en masse and when the process begins, lysosomes were stained with LysoTracker. Regardless of the cell death pathway, there is a subsequent increase in lysosomal activity that can be detected by LysoTracker (LT) dye (Fogel et al., 2012).

Dysgenic 1-day-old flies and 1st stage larvae were incubated at 20 °C and then transferred to 29 °C. The lysosomal activity in their gonads was monitored after 3 h, 1 day, and 3 days incubation at the restrictive temperature. In all variants, the LysoTracker-labelled area was wider in dysgenic progeny than in controls (n =100). In more detail, we have presented here an analysis of this effect in imago ovaries, because in this case, the pattern of lysosomal activity can be most clearly mapped relative to the inner structure of the ovary and easily quantified.

Lysosomal staining was observed in the ovaries of flies grown at 20 °C, as in dysgenic and control crosses, but did not exceed 20% of the germline cysts. Raising the temperature for 3 h resulted in an increase in the number of LysoTracker-stained areas in control flies around 15–20%, in region 2a/2b of the germarium and in mid-oogenesis. However, in dysgenic ovaries, an increase in the LysoTracker signal was detected in 95% of the ovarioles (n=500), occupying not only 2a/2b but also other regions of the germaria (Fig. 3A–G). In addition, lysis appeared in control ovaries locally and affected individual cysts. In dysgenic ovaries, only single cysts escaped death and could advance to the next stage of development. After a 3-day incubation at 29 °C, the germaria were considerably reduced due to the loss of not only individual germ cells but also the germaria regions (Fig. 3I and J). However, the germ cells revealed different sensitivity to the dysgenic effect and did not degrade at the same time, but for several days (Fig. 3H). Egg chambers of later stages, including vitellogenesis, exhibited only slight degradation in comparison to the control.

Enhanced lysosomal activity was also observed in testis, mainly in the areas occupied by early-stage cells (Fig. 3K and L).

3.4. Hybrid dysgenesis significantly affects the ultrastructure of germ cells and especially mitochondrial apparatus

We used electron microscopic analysis to characterize the cellular pathology associated with the massive lysis under conditions inducing HD. The gonads of adult dysgenic flies that had developed at 20 °C had an ultrastructure similar to that of the control. Signs of the ultrastructural pathological changes were first visible after 3 h of incubation of 1-day-old flies at 29 °C.

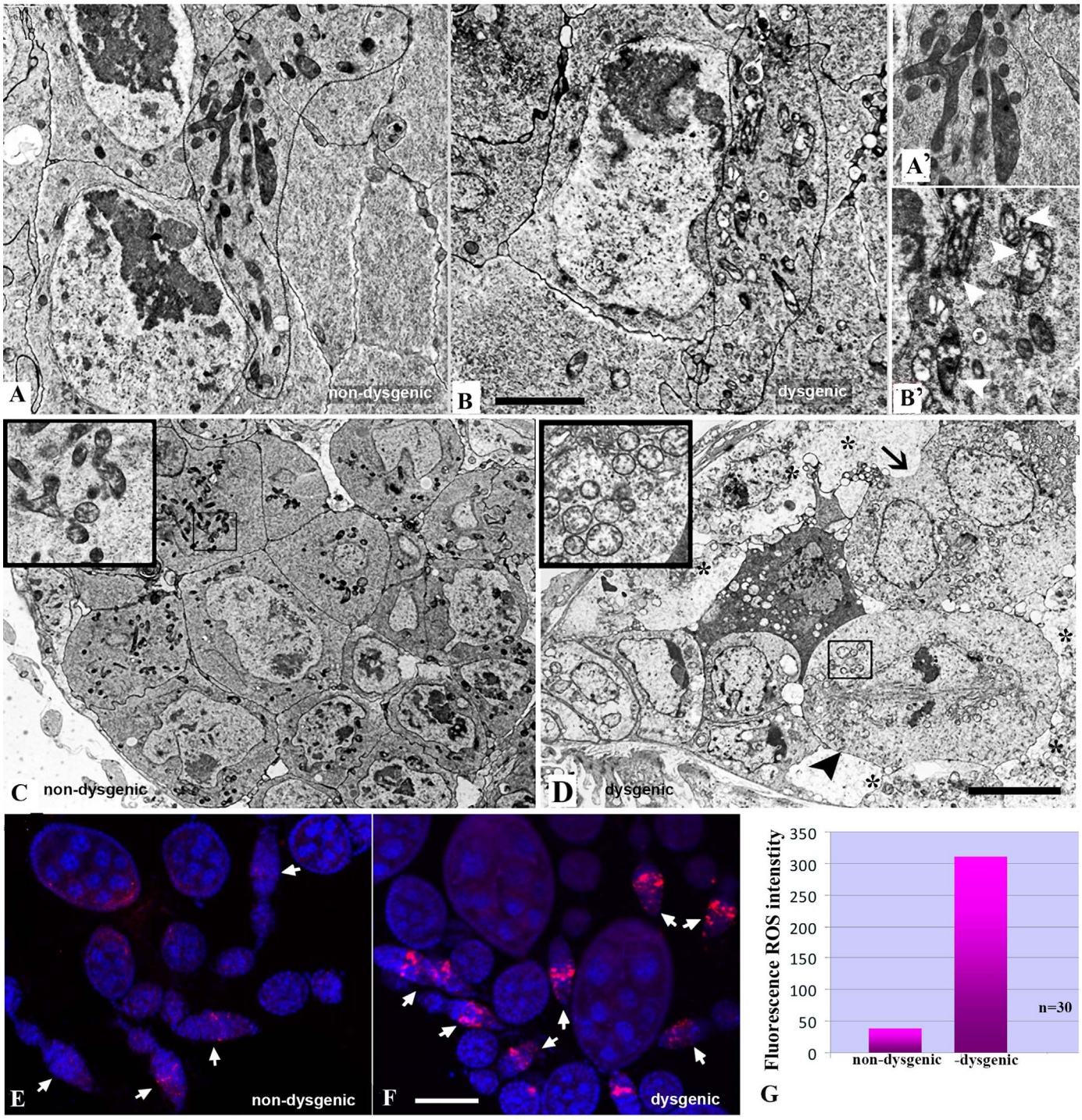


Fig. 4. Anomalies in fly imago gonadal internal structure caused by hybrid dysgenesis. A,B. Internal germ cell structure and mitochondrial morphology in germarium after a 3-h incubation at 29 °C. Compared with the control (A), the germ cells of dysgenic germaria contain mitochondria with altered ultrastructure (B). A′,B′. Dysgenic mitochondria (B′) show the features of initial swelling, they have areas with electron light matrix and are partially devoid of cristae (arrow). C,D. The ultrastructure of the 1–2 A germarium region in the control (C) and dysgenic (D) females after a 3-day incubation at the restrictive temperature. Dysgenic germaria have signs of intracellular structural degradation and cell death. However, some cells continue to divide despite the pathological changes. Fig. 3D demonstrates cells in metaphase (arrowhead) and anaphase (arrow). They form a normal cell division spindle and cleavage furrow, however the cytoplasm appears depleted and poorly granulated and mitochondria (highlighted in the square frame) are swollen and transparent. *- Area of cell cytoplasmic lysis. Two different experiments were carried. 10 control and 10 dysgenic flies were analyzed. E,F. The intracellular ROS accumulation in control (E) and dysgenic gonads (F) stained by CellROX® Deep Red. Dysgenic germaria display a strong increase in ROS content compared to control (arrows). DNA - DAPI (blue), ROS - CellROX® Deep Red (red). G. Comparative ROS fluorescence intensity in dysgenic and non-dysgenic germaria. Non-dysgenic– *Harwich/CantonS*, dysgenic - *CantonS/Harwich*. Scale bars: 1,5 μm (A–B), 1 μm (A′,B′), 4 μm (C–D), 30 μm (E,F).

After a 3-h incubation in females, in addition to increased number of lysosomes, we observed defects in mitochondrial morphology. In prefollicular germ cells, mitochondria are readily detectable in Bolbiani bodies where they pass through the fusome. Abnormal mitochondria of dysgenic ovaries were less structured, had areas with electron light matrix and partially lacked cristae (Fig. 4A, B, A′, B′).

Increasing the incubation time at 29 °C for 3 days was associated with a significant expansion of the lytic areas and a reduction in the

number of viable germ cells (Fig. 4C and D). This is consistent with the light microscopic data. Germ cells in dysgenic germaria showed an enhancement of the cytoplasmic defects. There were poorly granulated cytoplasm, swollen mitochondria with transparent matrix and depleted cristae (Fig. 4C, D and Supplementary material S1). However, some cells were able to form structures providing cell division, such as the division spindle and cleavage furrow (Fig. 4D). Ultrastructural analysis of dysgenic germaria (n=10) revealed that all germ cells show signs of degradation, but with varying degrees of manifestation (Supplementary material Fig. S1).

Dysgenesis induced anomalies in spermatogenesis phenotypically manifested as well in the oogenesis but to a lesser extent. We observed similar abnormalities in mitochondrial ultrastructure after a 3-h incubation of males at 29 °C, but compared with oogenesis they were not widespread. After a 3-day incubation at 29 °C, the mass death of cysts in the testis was evident but it was not as large-scale as in the ovaries (not shown).

Mitochondrial defects may be crucial for the cell viability if cause release of reactive oxygen species (ROS) into the cytoplasm. Increased ROS levels have cytotoxic effects and can initiate cell death (Ott et al., 2007). To confirm the causal connection between mitochondrial pathology and germ cells dying we studied intracellular pattern of ROS accumulation under dysgenic condition using CellROX® Deep Red dye. We analyzed CellROX staining at different time points and revealed that, after 5 h incubation at 29 °C, dysgenic germ cells demonstrated excessively high ROS level compared with the control (Fig. 4E–G). These data suggest that the germ cell fate in HD depends on quality of their mitochondria. Abnormal dysgenic mitochondria can generate unfavorable physiological conditions and lead to cell death.

3.5. Why are germ cells dying?

Programmed cell death is essential for normal germline development and plays a role in removing impaired or excess cells (Denton et al., 2013). In a typical *Drosophila* gonad, entire germline cysts undergo execution rather than single cells. In wild-type ovaries, germ cell death sporadically occurs after passing certain checkpoints within the germarium (region 2) and during mid-stages of oogenesis (stages 7–9) (McCall, 2004). During normal spermatogenesis, some of the spermatogonial cysts undergo spontaneous cell death (Yacobi-Sharon, et al., 2013). In addition, checkpoint control provides selective removal of cells with signs of unrepaired defects; however, in the case of HD, a massive loss of early germline cysts was detected in our analyses. These phenotypes are similar to those observed after the overexpression of the effector caspase Dcp-1 and downstream mediator Dmp53, which are important in cell death pathways (Barth et al., 2011). Therefore, it is possible that the uncontrolled germ cell death in dysgenic backgrounds is not associated with the checkpoint system.

The mechanism promoting germ cell degradation in HD is not clear. However, the sudden appearance of numerous defective mitochondria would lead to problems with cellular metabolism. Visually, the mitochondria are the very first subcellular structures that react to the effects of HD, and their internal structure is considerably degraded during the several hours of incubation at the elevated temperature. It is known that mitochondria accumulate not only dangerous reactive oxygen species but also some key regulators of cell death such as *cytochrome c* and other pro-apoptotic proteins (Krieser and White, 2009; Abdelwahid et al., 2011). Mitochondrial defects can disrupt the selective permeability of the outer membrane and result in the release of these factors into the cytosol, which triggers cell death. However, although we believe that the mitochondria are implicated in germline atrophy, it cannot be exclude that mitochondrial pathology are the consequence of activation of the cell death program triggered by other inducers. Therefore further studies are required to identify upstream regulators of cell death machinery and to define role for mitochondria in the process.

4. Conclusion

We investigated HD in the context of germ cell development and observed that these cells become susceptible to the dysgenic effect only after embryonic gonad formation. However, manipulating the incubation temperature can induce HD symptoms at any life-cycle stage of the dysgenic progeny, including in the imago. The gonads of adult dysgenic flies that had developed at 20 °C displayed a normal morphology and were completely filled with germ cells. However, the displacement of these flies to the restrictive 29 °C led to mitochondrial defects in germ cells and promoted their lysis and ultimate death. Quick and mass germ cell death creates difficulties in the analysis of the molecular mechanisms of HD, and particularly, experimental evidence of *P*-element activity in the induction of dysgenic syndrome. Our research has shown that the germcell lifetime can be controlled by means of temperature regulation. There is a lag-period between the beginning of HD induction and the onset of germ cell death. This time gap provides an opportunity to isolate the germline for molecular and cytological studies of *P*-element activity or other potential agents of HD.

Competing interests

The authors declare no competing or financial interests.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ydbio.2017.02.020.

References

Abdelwahid, E., Rolland, S., Teng, X., Conradt, B., Hardwick, J.M., White, K., 2011. Mitochondrial involvement in cell death of non-mammalian eukaryotes. Biochim. Biophys. Acta 1813 (4), 597–607.

Barth, J.M., Szabad, J., Hafen, E., Köhler, K., et al., 2011. Autophagy in Drosophila ovaries is induced by starvation and is required for oogenesis. Cell Death Differ. 18, 915–924.

Bhat, K.M., Schedl, P., 1997. Establishment of stem cell identity in the *Drosophila* germline. Dev. Dyn. 210, 371–382.

Denton, D., Aung-Htut, M.T., Kumar, S., 2013. *Developmentally programmed cell death in* Drosophila. Biochim. Biophys. Acta 1833, 3499–3506.

Dorogova, N.V., Fedorova, E.V., Bolobolova, E.U., et al., 2014. GAGA protein is essential for male germ cell development in *Drosophila*. Genesis 52, 738–751.

Engels, W.R., 1979. Hybrid dysgenesis in Drosophila melanogaster: rules of inheritance of female sterility. Genet. Res. 33, 219–236.

Engels, W.R., 1996. *P-elements* in Drosophila. Curr. Top. Microbiol. Immunol. 204, 103–123.

Engels, W.R., Preston, C.R., 1979. Hybrid dysgenesis in Drosophila melanogaster: The biology of female and male sterility. Genetics 92, 161–174.

Fogel, J.L., Thein, T.Z., Mariani, F.V., 2012. Use of LysoTracker to detect programmed cell death in embryos and differentiating embryonic stem cells. J. Vis. Exp. 11 (68), e4264.

Ignatenko, O.M., Zakharenko, L.P., Dorogova, N.V., Fedorova, S.A., 2015. P elements and the determinants of hybrid dysgenesis have different dynamics of propagation in *Drosophila melanogaster* populations. Genetica 143 (6), 751–759.

Kidwell, M.G., Novy, J.B., 1979. Hybrid dysgenesis in *Drosophila melanogaster*: Sterility resulting from gonadal dysgenesis in the P-M system. Genetics 92, 1127–1140.

Kidwell, M.G., Kidwell, J.F., Sved, J.A., 1977. Hybrid dysgenesis in *Drosophila melanogaster*: a syndrome of aberrant traits including mutation, sterility and male recombination. Genetics 86, 813–833.

Krieser, R.J., White, K., 2009. Inside an enigma: do mitochondria contribute to cell death in *Drosophila*? Apoptosis 14, 961–968.

Li, M.A., Alls, J.D., Avancini, R.M., Koo, K., Godt, D., 2003. The large Maf factor traffic Jam controls gonad morphogenesis in *Drosophila*. Nat. Cell Biol. 5 (11), 994–1000.
Majumdar, S., Rio, D.C., 2015. P transposable elements in *Drosophila* and other eukaryotic organisms. Microbiol. Spectr. 3 (2), (MDNA3-0004-2014).
Malone, C.D., Lehmann, R., Teixeira, F.K., 2015. The cellular basis of hybrid dysgenesis and stellate regulation in Drosophila. Curr. Opin. Genet Dev. 34, 88–94.
McCall, K., 2004. Eggs over easy: cell death in the *Drosophila* ovary. Dev. Biol. 274, 3–14.
Ott, M., Gogvadze, V., Orrenius, S., Zhivotovsky, B., 2007. *mitochondria, oxidative stress and cell death*. Apoptosis 12 (5), 913–922.
Patel, N., 1994. Imaging neuronal subsets and other cell types in whole mount *Drosophila* embryos and larvae using antibody probes. Methods Cell Biol. 44, 445–447.
Pertceva, J.A., Dorogova, N.V., Bolobolova, E.U., Nerusheva, O.O., Fedorova, S.A., Omelyanchuk, L.V., 2010. The role of Drosophila hyperplastic discs gene in spermatogenesis. Cell Biol. Int. 34, 991–996.
Schaefer, R.E., Kiddwell, M.G., Fausto-Sterling, A., 1979. Hybrid dysgenesis in *Drosophila melanogaster*: morphological and cytological studies of ovarian dysgenesis. Genetics 92, 1141–1152.
Yacobi-Sharon, K., Namdar, Y., Arama, E., 2013. Alternative germ cell death pathway in *Drosophila* involves HtrA2/Omi, lysosomes, and a caspase-9 counterpart. Dev. Cell 25 (1), 29–42.