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Article

Germ Cell Specification Requires Zygotic Mechanisms Rather Than Germ Plasm in a Basally Branching Insect

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Summary

Background: Primordial germ cell (PGC) specification is a universal process across animals, but the molecular mechanisms specifying PGCs are remarkably diverse. In *Drosophila*, PGCs are specified by maternally provided, asymmetrically localized cytoplasmic factors (germ plasm). In contrast, historical literature on most other arthropods reports that PGCs arise from mesoderm during midembryogenesis, suggesting that an arthropod last common ancestor may have specified PGCs via zygotic mechanisms. However, there has been no direct experimental evidence to date for germ plasm-independent arthropod PGC specification.
Results: Here we show that in a basally branching insect, the cricket *Gryllus bimaculatus*, conserved germ plasm molecules are ubiquitously, rather than asymmetrically, localized during oogenesis and early embryogenesis. Molecular and cytological analyses suggest that *Gryllus* PGCs arise from abdominal mesoderm during segmentation, and *twist* RNAi embryos that lack mesoderm fail to form PGCs. Using RNA interference we show that *vasa* and *piwi* are not required maternally or zygotically for PGC formation but rather are required for primary spermatogonial divisions in adult males.
Conclusions: These observations suggest that *Gryllus* lacks a maternally inherited germ plasm, in contrast with many holometabolous insects, including *Drosophila*. The mesodermal origin of *Gryllus* PGCs and absence of instructive roles for *vasa* and *piwi* in PGC formation are reminiscent of mouse PGC specification and suggest that zygotic cell signaling may direct PGC specification in *Gryllus* and other Hemimetabola.

Introduction

Of the many specialized cell types that comprise an animal's body, only one is capable of contributing genetic information to the next generation: the germ cells. The restriction of reproductive potential to a small subset of cells is a universal process across sexually reproducing animals and represents a profound evolutionary novelty likely required for the evolution of multicellularity [1]. The molecular mechanisms that specify these cells, however, are remarkably diverse between taxa [2–5] and only well understood in a handful of model organisms.

Primordial germ cell (PGC) specification mechanisms have been categorized into two modes: cytoplasmic inheritance and zygotic induction [3, 4, 6]. Cytoplasmic inheritance (e.g., in *Drosophila melanogaster*) involves the localization of maternal mRNAs and proteins (germ plasm) to a subcellular region of the oocyte. Germ plasm is necessary and sufficient to induce PGC fate. In zygotic induction (e.g., in *Mus musculus*), by contrast, there is no germ plasm, and PGCs instead form in response to inductive signals from neighboring somatic cells [7].

Within insects, cytoplasmic inheritance appears to be a derived character confined primarily to the holometabolous insects [8] (Figure 1A; see also Table S1 available online), where germ plasm has been demonstrated experimentally in many species (Table S1). Histological studies of insects branching basally to Holometabola (the Hemimetabola), in contrast, have reported the absence of both germ plasm and pole cells in nearly all of these taxa [3, 6] (Figure 1A; Table S1). Studies of molecular markers for PGCs in hemimetabolous insects have been limited to the highly atypical parthenogenetic embryos of the pea aphid *Acyrthosiphon pisum*, a milkweed bug, and several orthopteran species (Table S1), yet there is no conserved pattern of PGC origin across these taxa.

In this study, we use multiple conserved molecular markers and RNAi to characterize PGC formation in the cricket *Gryllus bimaculatus* (Orthoptera), a hemimetabolous model species for studying the development of basally branching insects [9]. We provide several lines of evidence that *Gryllus* PGCs form from the abdominal mesoderm via inductive signaling and discuss the implications of these results for the evolution of germ plasm and the possibility of an ancient relationship between bilaterian PGCs and mesoderm.

Results

Gryllus Germ Cells Express a Suite of Conserved Genes

Within the Orthoptera, neither germ plasm nor pole cells have been reported (Figure 1A; Table S1). Histological examinations of orthopteran embryos conducted by William Wheeler over a century ago [10] suggested that PGCs arise from or among abdominal mesoderm cells during abdominal segmentation (Figure 1C), consistent with reports of germline origin both in other Hemimetabola and in most arthropods [3, 6]. However, conserved molecular markers can reveal a cryptic germ plasm that eludes histological examinations [11–13]. We therefore examined the expression of several conserved molecular PGC markers (*vasa* [14], *piwi* [14], *tudor*, *boule*, and *germ cell-less*) and three additional PIWI family genes (Figures S1A and S1B) in *Gryllus* ovaries and embryos. Because some germ plasm components localize as proteins rather than transcripts (see for example [15, 16, 17]), we also examined the expression of Vasa and Piwi proteins [14].

In fully segmented (stage 9) *Gryllus* embryos (Figures 1B and 1C), we identified cells matching Wheeler's description [10] that express both mRNA and protein of *piwi* and *vasa*, as well as *bol* and *gcl* transcripts (Figures 1D, 1E, and S1C). These cell clusters were found in abdominal segments A2–A3 in all embryos, and in A4–A5 in 45% of embryos (Figures 1D and 1E, arrowheads). Clusters were located on the dorsal medial face of mesodermal structures termed "coelomic pouches," which are present in every gnathal, thoracic, and abdominal

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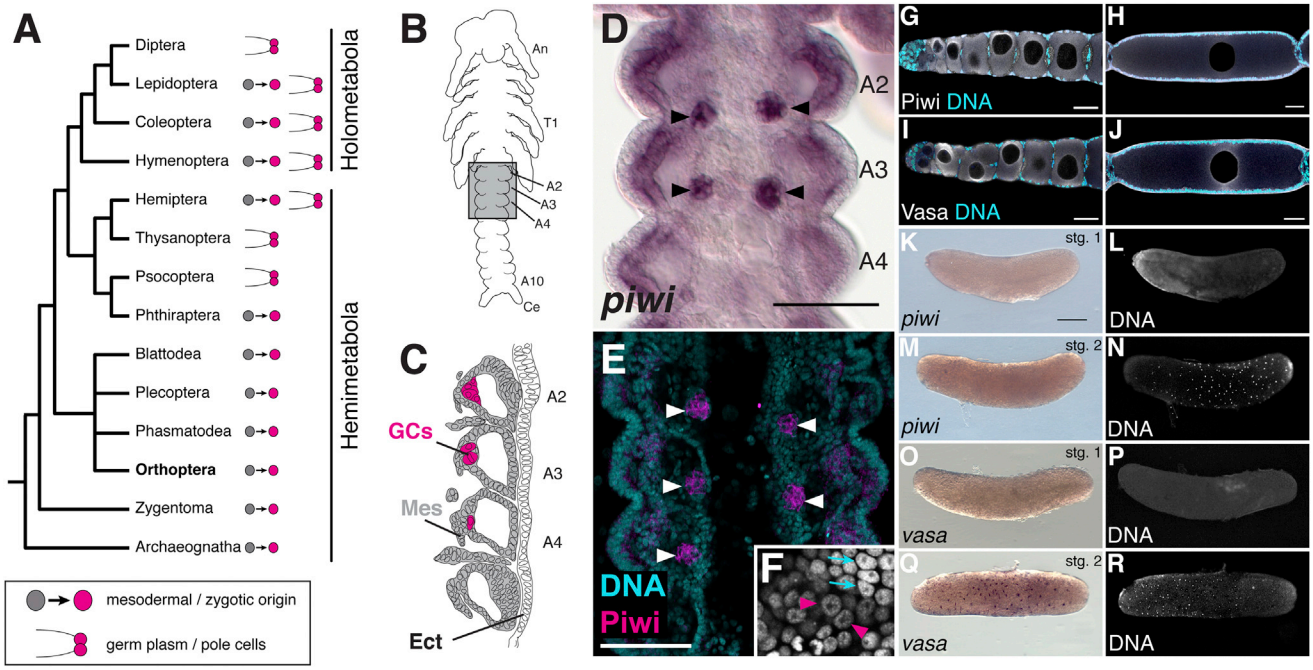

Figure 1. Molecular Markers Suggest Absence of Germ Plasm in *Gryllus*

(A) Phylogenetic distribution of reported PGC specification mechanisms across insects (see Table S1).
(B) Schematic of a stage 9 *Gryllus* embryo, highlighting the region enlarged in (D)–(E) (gray box). A2–A4, abdominal segments 2–4.
(C) Tracing of Wheeler's description of orthopteran PGCs at the earliest time point they could be identified [10]. GCs, germ cells (magenta); Mes, mesoderm (gray); Ect, ectoderm (white).
(D and E) *Gryllus* PGCs (arrowheads) express *piwi* transcripts (D) and protein (E).
(F) PGCs (arrowheads) display nuclear morphology distinct from somatic cells (arrows).
(G–J) Piwi (G and H) and Vasa (I and J) proteins do not localize asymmetrically in the ooplasm.
(K and M) *piwi* transcripts are undetectable during stages 1–2.
(O and Q) *vasa* transcripts are undetectable at stage 1 (O) and associated with all energid nuclei at stage 2 (Q).
(L, N, P, and R) Corresponding nuclear stains.
Scale bar represents 100 μm in (D) and (E); 50 μm in (G)–(J); 200 μm in (K)–(R). Anterior is up in (B)–(F), left in (G)–(R). See also Figures S1, S2, and Table S1.

segment. These cells possessed universal PGC characteristics [3] of large nuclei with diffuse chromatin and a single large nucleolus (Figure 1F). Based on these gene expression, nuclear morphology, and embryonic location data, we conclude that these cells are *Gryllus* PGCs. We also examined the expression of four additional putative candidate PGC marker genes (*tudor*, *piwi-2*, *AGO3-A*, and *AGO3-B*) but found that they were not specific PGC markers in *Gryllus* embryos (Figures S1B and S1F).

Gryllus Germline Markers Do Not Localize within Oocytes or Reveal PGCs in Early Embryos

We next examined the expression of *Gryllus* PGC markers during earlier stages of embryogenesis and oogenesis to test whether they revealed the presence of germ plasm in oocytes or PGCs in early embryos. All genes tested were consistently ubiquitous throughout oogenesis and never localized asymmetrically within the ooplasm (Figures 1G–1J, S1D, and S1F), although Vasa and Piwi proteins were enriched around the oocyte nucleus (Figures 1G–1J). In blastoderm-stage embryos (stages 1–3) and early germband-stage embryos (stage 4), *piwi* (Figures 1K–1N, S1E, and S2G–S2P′), *vasa* (Figures 1O–1R, S1E, and S2Q–S2Z′), *bol*, and *gcl* (Figures S2A–S2F) were expressed ubiquitously at low levels and showed no asymmetric localization within the embryo. These results are in stark contrast to the posterior accumulation of PGC determinants in *Drosophila* oocytes and early embryos [16, 18–20] and suggest an absence of germ-plasm-driven PGC specification in *Gryllus*.

Gryllus PGCs Arise De Novo during Midembryogenesis

To determine the embryonic origin of *Gryllus* PGCs, we examined the expression of *piwi* and *vasa* transcripts and proteins throughout abdominal elongation and segmentation. During early germband stages (stage 4), we detected low-level ubiquitous expression of both genes in all ectodermal and mesodermal cells (Figures 2A–2B′ and S3A–S3B′). It was not until thoracic limb bud enlargement began (stage 5) that *piwi* transcripts were detected at higher levels in two subsets of cells in abdominal segments A2–A4 among the lateral abdominal mesoderm (Figures 2C and 2C′). As appendage elongation began (stage 6), *piwi*-positive cells split into distinct groups along the anterior-posterior axis (Figures 2E and E′), and Piwi protein levels rose in these cells (Figures 2E″ and 2E‴). During morphological segmentation of the abdomen (stages 7–9) these cell groups coalesced into four to six distinct clusters adjacent and dorsal to the coelomic pouches in segments A2–A4 and continued to express high levels of *piwi* transcripts and protein (Figures 2F–2H‴ and S3D–S3F‴). *vasa* transcript and protein expression was similar to that of *piwi*, but *vasa* became enriched in PGCs slightly later than *piwi* and showed higher expression levels in the soma (Figure S3).

Interestingly, hallmarks of active transcription were observed in PGCs throughout all stages examined (Figures

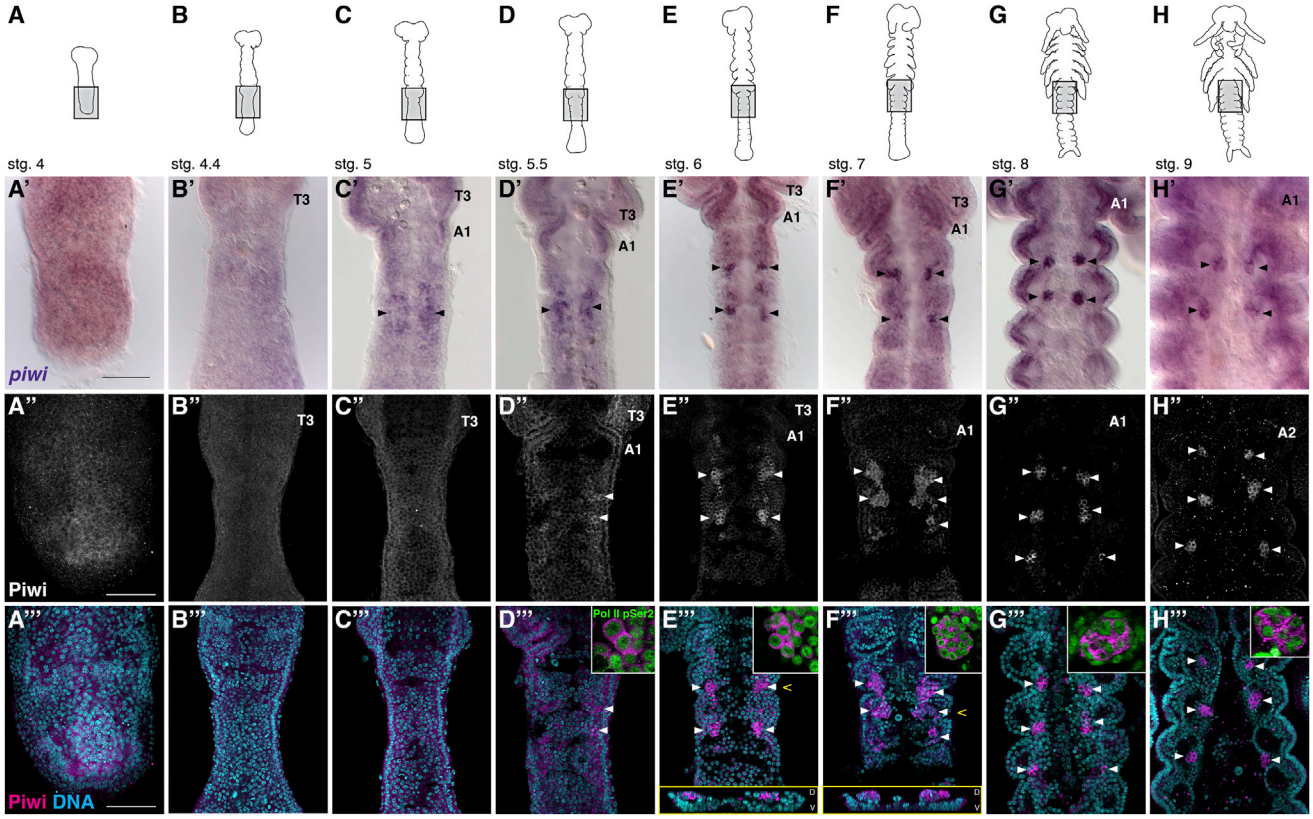

Figure 2. *Gryllus* PGCs Arise during Early Segmentation Stages

Drawings of *Gryllus* embryogenesis (A–H), highlighting the region depicted below (gray boxes). *piwi* transcripts are ubiquitous in stages 4 and 4.4 embryos (A′ and B′), but beginning at stage 5 (C′), two bilateral groups of *piwi*-positive cells arise on the dorsal surface of the embryo, then resolve into clusters during later stages in abdominal segments A2–A4 (D′–H′). Piwi protein shows a similar expression pattern to that of *piwi* transcript but is enriched slightly later in development (A″–H″). Piwi (magenta) overlaid on nuclear stain (cyan) reveals that PGCs arise prior to coelomic pouch formation and ultimately reside medial to these mesodermal structures (A‴–H‴). Yellow-framed insets in (E‴) and (F‴) show orthogonal projections at the position of the caret, illustrating the dorsal location of PGCs. Insets in (D‴)–(H‴) show expression of RNA polymerase II pSer2 (green) in PGCs. T3, thoracic segment 3; A1 and A2, abdominal segments 1 and 2.

Scale bar represents 100 μm. Anterior is up in all panels. See also Figures S2 and S3.

2D‴–2H‴, insets). This is consistent with *Gryllus* PGC formation via active transcriptional response to inductive signaling between cells rather than PGCs being a transcriptionally quiescent subpopulation of early-segregated cells as seen in *Drosophila* and other species with germ plasm [21].

Consistent with a conversion of presumptive mesoderm cells to PGCs beginning at stage 5, the nuclear morphology of mesodermal cells correlated with the relative levels of Piwi expression throughout development. At stage 4, all mesoderm cells had uniform Piwi expression and nuclear morphology, relatively compact chromatin, and multiple nucleoli (Figures 3A and 3A′). As Piwi expression increased in presumptive PGCs, their nuclei became larger with increasingly diffuse chromatin, whereas nuclei of neighboring Piwi-poor cells decreased in size, and their chromatin became compact as they progressed through mesoderm differentiation (Figures 3B–3D′). By stages 8–9, PGCs were clearly distinguished by their high nuclear-cytoplasmic ratio, diffuse chromatin, and single large nucleolus (Figures 3E–3F′), criteria used to identify PGCs in historical studies of Orthoptera and other animals [3]. Following stage 10, PGC clusters merged via short-range cell migration (Figure 3G) and coalesced into two bilateral gonad primordia (Figure 3H) located in segments A3–A4. Thus, *Gryllus* PGCs do not undergo long-range migration, as they do in many other species including *Drosophila* [22], but rather arise near the location of the embryonic gonad.

Knockdown of *Gryllus piwi* or *vasa* Does Not Disrupt PGC Formation or Maintenance

We knocked down *vasa* and *piwi* function using both maternal and zygotic RNAi (mRNAi and eRNAi, respectively) and confirmed knockdown using qPCR and immunostaining (Figures 4A, 4B, 4E, and 4H). In contrast to *Drosophila*, in which *vasa* and *piwi* are required maternally for embryonic PGC formation [15, 18], mRNAi against *vasa* and *piwi* did not disrupt PGC formation in *Gryllus* embryos (Figures 4C–4H), and there was no significant difference in the number of PGCs in either *vasa* or *piwi* mRNAi or eRNAi embryos relative to controls (Figures 4I and 4J). Furthermore, female embryos laid by mothers injected with *vasa* or *piwi* double-stranded (ds)RNA ultimately grew into fertile adults with fully functioning ovaries (Figure 4K–4M). In contrast to the *Drosophila* requirement for *vasa* and *piwi* in oogenesis and axial patterning [15, 23], *Gryllus* females injected with *vasa* or *piwi* dsRNA displayed no defects in egg laying, oogenesis, or axial patterning (Figures S4A–S4C). Moreover, double knockdown of *vasa* + *piwi* maternally or zygotically did not

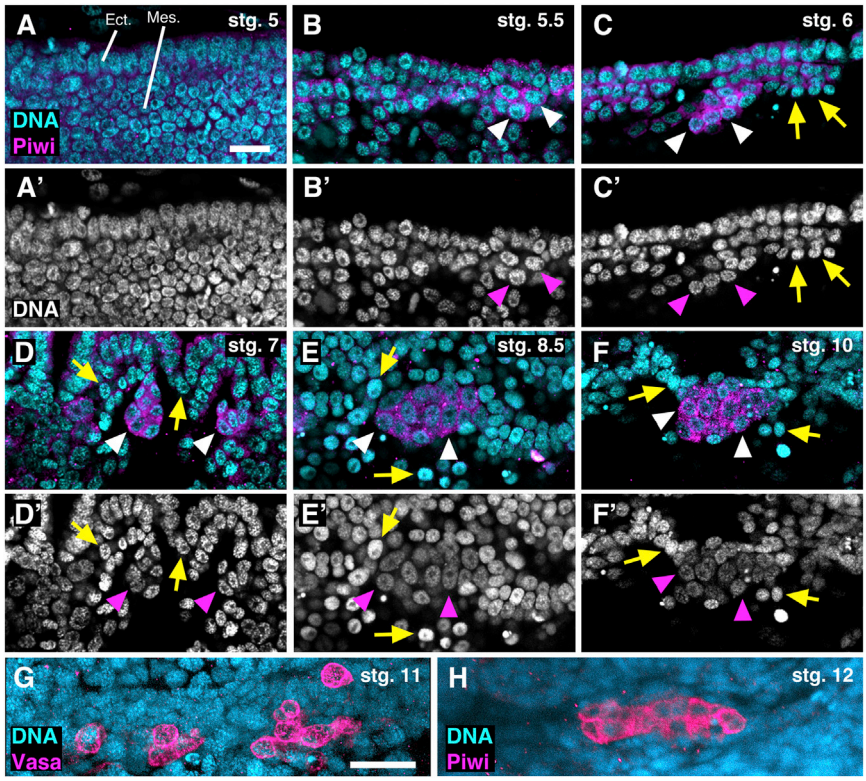

Figure 3. Piwi Expression Correlates with Acquisition of PGC Nuclear Morphology in Abdominal Mesoderm Cells

(A–F′) Between stages 5 and 10, Piwi expression increases in PGCs (A–F, arrowheads), and nuclear morphology of Piwi-enriched cells changes accordingly (A′–F′, arrowheads). Late stage 5 PGCs have chromatin compaction and multiple nucleoli similar to neighboring mesodermal cells (B and B′). As Piwi enrichment in PGCs increases (arrowheads), their chromatin becomes more diffuse and nuclear size increases (yellow arrows) (C and C′). In subsequent stages chromatin morphology differences become more pronounced (D–F′).

(G and H) At stage 11 (G), PGCs commence short-range migration along the anterior-posterior axis toward the intersegmental region of A3–A4 to form a single gonad primordium on each side of the embryo by stage 12 (H).

Scale bars represent 50 μm in (A) (applies to A′–F′) and (G) (applies to H). Anterior is to the left.

disrupt PGC formation or axial patterning (Figures S4C–S4E), indicating that these genes do not act redundantly to direct PGC specification.

vasa and *piwi* Play Roles in *Gryllus* Spermatogenesis

In mice, which lack germ plasm and specify PGCs from presumptive mesoderm via signaling, *vasa* and *piwi* are not required for PGC specification but do mark established PGCs of both sexes and play roles in adult spermatogenesis [24, 25]. We tested whether these genes were required for adult spermatogenesis in *Gryllus* by injecting adult males with dsRNA for *vasa* or *piwi* to achieve paternal RNAi (pRNAi). *Gryllus* testes comprise 200–300 testioles (sperm tubules) [26], within which spermatogenesis proceeds from anterior to posterior (Figures 5A and S5A–S5G). The anterior region of each testiole expresses Vasa and Piwi proteins (Figures S5T and S5U) and contains primary and secondary spermatogonia (Figure 5A). Knockdown of *vasa* or *piwi* via pRNAi severely reduced spermatogonial region length (Figure 5H). In both *vasa* and *piwi* pRNAi testes, meiotic spermatocytes were found in the anterior region of testioles, in some cases almost abutting primary spermatogonia (Figures 5C, 5D, 5F, and 5G, yellow arrowheads), and secondary spermatonial cysts were reduced (Figures 5C, 5D, and 5G, red arrows) or absent (Figure 5F), suggesting that the mitotic divisions of primary spermatogonia were affected. The misregulation of primary spermatocyte divisions was not due to absence of the germline stem cell niche (apical cell), which was present in *piwi* and *vasa* pRNAi testes (Figures 5E–5G, asterisks, and S5B, S5H, and S5N). Postspermatogonial stages of spermatogenesis appeared unaffected (Figures S5I–S5M and S5O–S5S). These data indicate that, as in mice and other animals (see Discussion), *piwi* and *vasa* play a role in *Gryllus* gametogenesis in adult males.

Mesoderm Is Required for *Gryllus* PGCs

Our observations thus far suggested that PGCs arise from among mesodermal cells during abdominal segmentation. To test this hypothesis, we took advantage of the conserved role of the *twist* gene in mesoderm development [27] to ask whether PGCs could form if mesoderm development was compromised. *Gryllus twist* is expressed in the abdominal mesoderm beginning during axial elongation, including in cells of the region where PGCs arise (Figures S6A–S6D2′). In *Drosophila*, *twist* mutants display gastrulation defects [28], yet PGCs form normally because PGC specification occurs via germ plasm well before gastrulation (Figures 6A and 6E). In *Gryllus*, *twist* eRNAi similarly causes disorganization or loss of major mesodermal structures within all body segments (Figures 6F and 6G, compare to 6B and 6C). In contrast to *Drosophila*, however, 49% of *Gryllus twist* eRNAi embryos lack PGCs, compared to 0% of controls ($p < 0.01$, Figures 6D and 6G′), and those *twist* eRNAi embryos that specify PGCs have fewer than controls ($p = 0.05$, Figure 6H). These results are consistent with the hypothesis that PGCs form from a subset of abdominal mesoderm. Alternatively, PGCs may be formed normally at stage 5 but fail to be maintained due to absent or compromised mesodermal surroundings.

Discussion

We have shown that neither *vasa* nor *piwi* are required maternally or zygotically for the formation of functional PGCs (Figures 4 and S4) but instead play a role in spermatogonial divisions in adult males. Our results differ from those of analogous experiments in *D. melanogaster* [15, 18], indicating that the functions of these genes have diverged between *Gryllus* and *Drosophila*. Although these genes are not required for *Gryllus* PGC formation, we propose that, together with *gcl* and *boule* expression (Figure S2) and the transition from mesodermal to PGC-like morphology in situ (Figure 3), *vasa* and *piwi* are nevertheless informative *Gryllus* PGC markers, despite their pleiotropic roles in other developmental

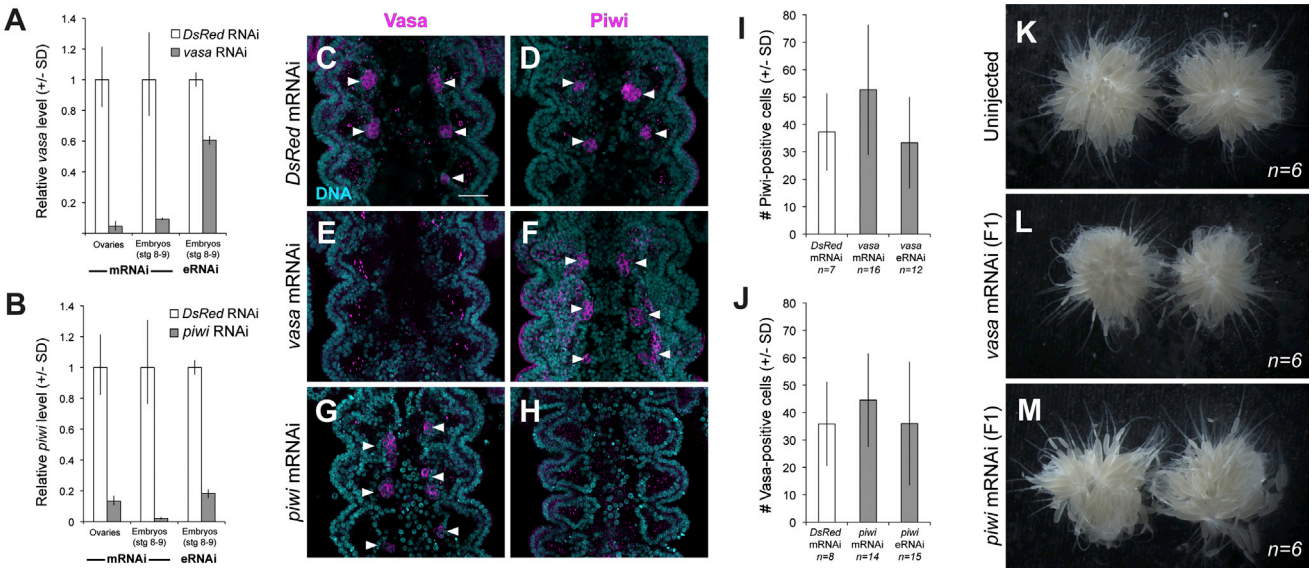

Figure 4. *vasa* and *piwi* Are Not Required for *Gryllus* PGC Specification

(A and B) qPCR validation of *vasa* and *piwi* knockdown following mRNAi and eRNAi.

(C–H) Piwi-positive PGCs (arrowheads) form in *vasa* RNAi embryos, and Vasa-positive PGCs form in *piwi* RNAi embryos. Consistent with qPCR results, *vasa* mRNAi (E; 100%, n = 9) and *piwi* mRNAi (H; 60%, n = 10) abolished respective protein expression. eRNAi produced similar results (not shown).

(I and J) PGC quantification confirms that PGC formation is not reduced (Student's t test: *vasa* mRNAi p = 0.07; *vasa* eRNAi p = 0.57; *piwi* mRNAi p = 0.24; *piwi* eRNAi p = 0.77).

(K–M) Ovaries from adult offspring of *vasa* and *piwi* pRNAi mothers (L–M) are indistinguishable from uninjected controls (K). Scale bar represents 50 μm in (C)–(H).

See also Figure S4.

processes. We cannot eliminate the possibility that untested marker genes might show an earlier PGC specification event than the one we identify in stage 5 (Figure 2C). However, given the conserved coexpression of the tested genes in PGCs of multiple metazoans, we believe it unlikely that all four would be absent from *Gryllus* PGCs at the time of their specification.

Evidence from multiple systems suggests that functional divergence of *vasa* and *piwi* is widespread. In

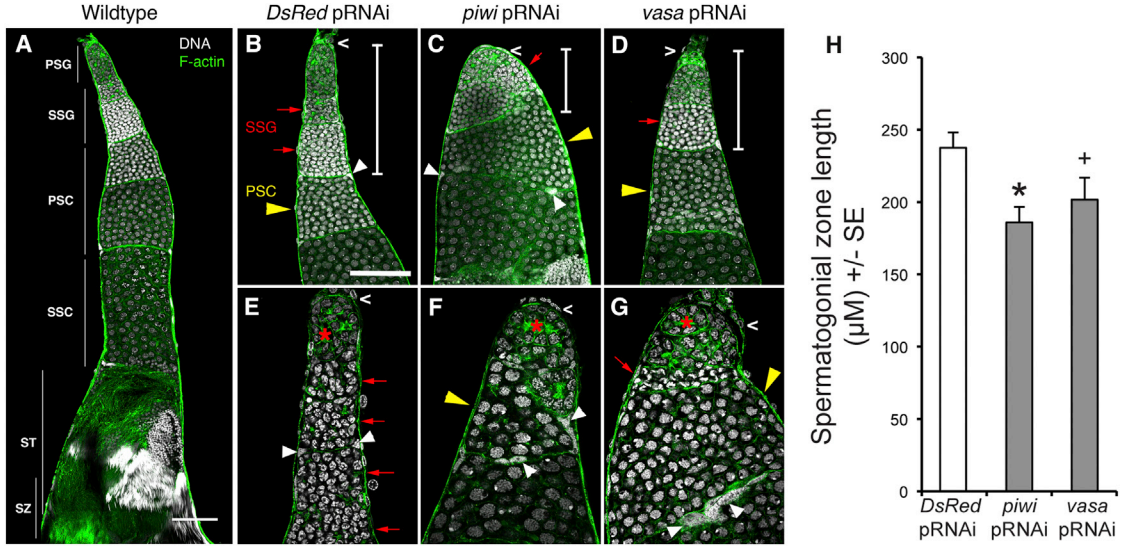

Figure 5. *piwi* and *vasa* pRNAi Causes Defects in Spermatogonial Proliferation

(A) Wild-type *Gryllus* testiole showing the stages of spermatogenesis.

(B–G) White bars in (B)–(D) indicate the spermatogonial zone containing secondary spermatogonia (SSG, red arrows). The zone of primary spermatocytes (PSC, yellow arrowheads) nearly abuts the primary spermatogonial zone in *piwi* (C and F) and *vasa* (D and G) pRNAi testes because of the shortened SSG zone but is absent from the anterior region of control testioles that have extensive SSG populations (B and E). Higher magnification (E–G) is shown of anterior testiole regions in control (E), *piwi* RNAi (F), and *vasa* RNAi (G) testes.

(H) *vasa* or *piwi* paternal RNAi results in a shortened spermatogonial zone compared to controls (Student's t test: *p < 0.01, +p = 0.06).

Scale bar represents 100 μm in (A) and 50 μm in (B) (applies also to C–G). Anterior is up in (A)–(G). See also Figure S5.

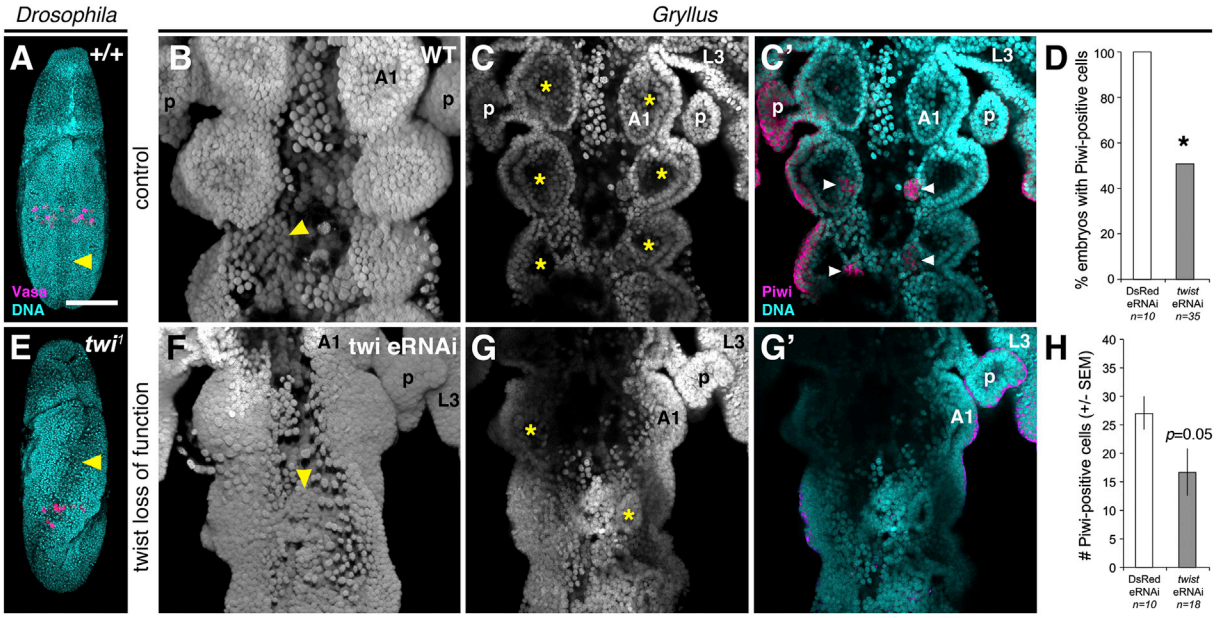

Figure 6. *twist* eRNAi Disrupts *Gryllus* PGC Formation

Drosophila twist^{1} embryos display gastrulation defects (A and E; arrowhead indicates ventral furrow) but specify PGCs (magenta) properly. In comparison to wild-type (B–C′), *Gryllus twist* eRNAi embryos (F–G′) have disorganized mesoderm (F, arrowhead), show absent or rudimentary coelomic pouches (G, asterisks), and fail to specify PGCs (G′) significantly more often than controls (D; Fisher's exact test, *p < 0.01). When they do specify PGCs, *Gryllus twist* eRNAi embryos have fewer PGCs than controls (H; Student's t test, p = 0.05).

Scale bar represents 50 μm and applies to all panels. L3, third thoracic leg; A1, first abdominal segment; p, pleuropodia. Anterior is up. See also Figure S6.

D. melanogaster, where both genes were first discovered, mutations in *vasa* or either of the two *piwi* orthologs (*piwi* and *aubergine*) cause defects in germ plasm formation, oogenesis, PGC specification, and posterior patterning [15, 18, 19, 29]. Similarly, *vasa* and *piwi* orthologs are required for PGC specification, development, and oogenesis in *C. elegans*, *D. rerio* [see 2], and medaka [30]. In mice, however, *vasa* is expressed in embryonic PGCs of both sexes, but *vasa*$^{-/-}$ homozygotes display no discernable defects in PGC specification or oogenesis and instead show a male-specific defect in spermatogenesis [25]. Similarly, knockout mice for any of the three PIWI family homologs display spermatogenic defects only, with no defects in females [24, 31, 32]. Our data therefore suggest that the roles of *Gryllus vasa* and *piwi* are similar to those of their mouse homologs. Functional genetic and gene expression data from insects (Table S1) suggest that, in this clade, an instructive role for these genes in PGC formation may be restricted to the Holometabola, perhaps concomitant with the co-option of *oskar* to the top of the PGC specification pathway [14]. Consistent with this hypothesis, *vasa* is dispensable for PGC formation in another hemimetabolous insect, the milkweed bug, *Oncopeltus fasciatus* [33].

Our data indicate that a zygotic mode of PGC specification is likely present in *Gryllus*, whereby PGCs appear to arise from presumptive mesoderm. Because *twist* is expressed broadly in mesodermal cells (Figure S6), our *twist* RNAi results could indicate either that mesoderm gives rise to PGCs directly or that mesoderm is required to maintain PGCs (we note that these interpretations are not mutually exclusive). However, our morphological (Figure 3) and gene expression (Figures 2, S3, and S6) analyses strongly suggest that cells convert from mesodermal to PGC fate in situ. Alternatively, an undifferentiated population of PGC precursors could exist that do not express any of the tested PGC marker genes but are induced to adopt PGC fate by adjacent mesodermal cells. If this is the case, however, we note that such pluripotent precursors cannot require maternal provision of *vasa* or *piwi* and would most likely be specified by zygotic mechanisms.

Several lines of evidence suggest that a cell lineage relationship between mesoderm and the germline may be a cell type association predating the emergence of Bilateria. Bilaterian germ cells are strikingly similar in gene expression and cytological characteristics to endomesodermally derived stem cells in bilaterian outgroups. Whereas nonbilaterians do not have a dedicated germline per se, their pluripotent stem cell populations serve the function of the germline (reviewed in [34]), and cnidarian pluripotent stem cells are derived from endomesoderm during embryogenesis [35–39]. Within bilaterians, gametogenic cells are consistently described as arising from gonadal epithelia of mesodermal origin in most arthropods and many marine invertebrates (reviewed in [3]). In many spiralians, cytological, cell lineage, and molecular data indicate that PGCs originate from a multipotent mesodermal precursor or precursors (see also [3, 40–47]). Recent studies suggest that mouse PGCs may default to a mesodermal specification program if germline induction signals are absent [48, 49]. The work presented here illuminates broad similarities between PGC specification and *vasa* function in *Gryllus* and in the mouse. Future work will be required to explore this apparent similarity in greater depth and to determine the extent of conservation in the developmental and molecular processes involved in specifying the germline across Bilateria.

Experimental Procedures

Gryllus husbandry, gene expression analysis, mRNAi, eRNAi, and phenotypic analysis were carried out as previously described [50]. For pRNAi, 5 μl of 3 μg/ml dsRNA was injected into the coelomic cavity of adult males

1–3 days after the final molt to sexual maturity, and testes of injected males were dissected for analysis 7 days after injection (details in Supplemental Experimental Procedures).

Accession Numbers

Sequences have been deposited in GenBank (accession numbers KC242803–KC242808).

Supplemental Information

Supplemental Information includes six figures, Supplemental Experimental Procedures, and one table and and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2013.03.063.

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