Focus	Development of cryopreservation method of Drosophila strains
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Period	FY2012 - 2013
Overview	The National BioResource Project “Drosophila” (NBRP Drosophila) is conducted by consortium composed of five research institutes and universities that function as one of the largest Drosophila stock centers in the world. Despite the various efforts made historically, efficient techniques for cryopreservation of Drosophila stocks have not been established. By contrast, along with recent years’ development of genome information and genetic engineering technology, large-scale, systematic mutant production projects are ongoing. The reality is that only a limited number of stock centers exist worldwide and are struggling for Drosophila stock preservation. This program aims to develop a cryopreservation technique for Drosophila stocks. While the cryopreservation of ova (embryos) or sperm has been attempted so far, the preservation of larval ovaries is to be targeted in this program. The larval ovary is connected to the oviduct extended from the external genital anlage during metamorphosis and then forms a functional reproductive system in mature females. Thus, a certain percentage of ovaries transplanted during the larval stage may be incorporated into the reproductive system, followed by mature egg-deposition. Female germ cell differentiation does not yet occur in the ovaries even in mature larvae, and so high tolerance to cryopreservation is expected. A first-year goal is technical development to enhance the ovarian transplantation efficiency up to a practical level. It includes the improvement in a manufacturing method for glass needles for transplantation and testing on the usefulness of host larvae without ovaries. A technique for ovary cryopreservation is scheduled to be exploited during the second year.
Progress	(written in Japanese) 1) 幼虫卵巣移植技術の確立 : Needle Puller、Micro Forge、Needle Grinder、それぞれの至適条件を検討し、50um 径の終令幼虫卵巣の移植に最適な狭窄付の鋭利なガラス針を作成。 幼虫 154 個体への移植から 53 匹（34%）の成虫が羽化し、妊性のある成虫 47 匹中 14 匹（30%）から移植卵巣由来の F1 個体（計 620 匹）が羽化するまでの結果を得ることができた。 最終的な移植成功率は 10%弱となるが、とりあえず系統保存手段として検討可能なレベルと考える。 2) 卵巣凍結法の確立 : カイコ蛾卵巣の凍結条件を元に凍結保護剤処理時間、凍結時間等の検討を様々に試みた。 卵巣に GFP を発現する系統を用い、また vital staining で凍結融解後の卵巣細胞生存条件を検討したところ、negative control に比較してわずかに陽性のシグナルが検出されるまでになっている。 より適した条件を探索すると共に、生体への移植による検討が必要である。 発表 2012 年 12 月 日本分子生物学会特別展示 ポスター 2013 年 12 月 日本分子生物学会特別展示 ポスター